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ABSTRACTS FROM THE ORIGINAL PAPERS.

The Constitution of Kojic Acid, a γ-Pyrone Derivative Formed by Aspergillus Oryzae from Carbohydrates.

By T. YABUTA.

Previous work (J. Chem. Soc. Tokyo, 1916, **37**, 1185, 1234) has shown that kojic acid (I) is probably the alcohol corresponding with comenic acid. Interconversion by oxidation of kojic acid or reduction of comenic acid has not been accomplished, but both compounds furnish the same *1* : *5*-dihydroxy-2-methylpyridine. Kojic acid on treatment with thionyl chloride yields *5*-hydroxy-2-chloromethyl-γ-pyrone, needles, m. p. 119-121°, and on reduction with zinc dust and acetic acid gives allomaltol (*5*-hydroxy-2-methyl-γ-pyrone), prisms, m. p. 166°. Allomaltol is distinct from maltol (Brand, Ber., 1894, **27**, 806) and from isomaltol (Backe, Compt. rend., 1910, **150**, 540; **151**, 78), the distinction being confirmed by the preparation of the following derivatives. Allomaltol methyl ether (*5*-methoxy-2-methyl-γ-pyrone), prisms, m. p. 70–71°, b. p. (approx.) 93°/0.1 mm.; benzoylallomaltol (*3*-benzoyloxy-*6*-methyl-γ-pyrone), prisms, m. p. 128–129°; phenylcarbamate of allomaltol, NHPh. CO. $C_6H_5O_3$, plates, m. p. 186–188°; bromoallomatol (*6*-bromo-*5*-hydroxy-2-methyl-γ-pyrone), crystals, m. p. 171–173°. Benzene azoallomaltol (*6*-benzeneazo-*5*-hydroxy-2-methyl-γ-pyrone), by treating allomaltol in solution with diazobenzene acetate, forms reddish-brown needles, no definite m. p.

Oxidation of the following kojic acid derivatives did not give the corresponding comenic acid derivatives: monobenzoyl kojic acid; monoethyl ether of kojic acid, (*5*-ethoxy-2-hydroxy-methyl- γ-pyrone), prepared by the interaction of kojic acid, ethyl *p*-toluenesulphonate, and sodium ethoxide in alcohol, needles, m. p. 110°; *5*-ethoxy-2-hydroxymethyl-4-pyridine, prepared from kojic acid monoethyl ether, and isolated as the picrate needles, m. p. 184–185°; *5*-hydroxy-2-

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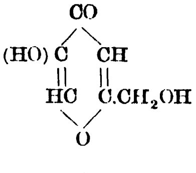
chloromethyl-γ-pyrone and its methyl ether; *5*-hydroxy-2-iodomethyl-γ-pyrone, prepared from the corresponding chloro-compound as crystalline plates; *5*-methoxy-*2*-iodomethyl-γ-pyrone, prisms, m. p. 135- 137°.

The following derivatives of comenic acid were prepared, but reduction did not give the corresponding kojic acid derivatives. Benzoylcomenic acid, prisms from alcohol, m. p. 227–228°; methyl ether of comenic acid, prisms, m. p. 280–282°; methyl ether of methyl comenate, m. p. 197°; methyl ether of ethyl comenate, m. p. 156°; the hydrochloride of the methyl ether of comenyl chloride (II), prepared by the action of thionyl chloride on comenic acid methyl ether, prisms, m. p.103°: the methyl ether of comenyl chloride, prepared from the previous compound by the action of sodium acetate, m. p. 148°: comenamide methyl ether, prepared by the action of ammonia on (II), needles, m. p. 178°. The catalytic reduction of the methyl ether of comenyl chloride by Rosenmund's method (Ber., 1918, **51,** 585; 1921, **54,** (B), 425, 638), failed owing to the instability of the pyrone nucleus. Comenamic acid methyl ether (*5*-methoxy-*4*-pyridone-2-carboxylic acid) was prepared by the action of ammonia on comenic acid methyl ether; ammonium salt, needles, m. p. 265°; hydrochloride, prisms. Thionyl chloride introduces chlorine into the *4*-position of the methyl ether, giving *4*-chloro-*5*-methoxypyridine-2-carboxylic acid chloride, which Rosenmund's method also failed to reduce. The acid chloride was converted into *4*-chloro-*5*-methoxy-pyridine-2-carboxylic acid, prisms, m. p. 209°, into the amide, needles, m. p. 207–208°, into the ethyl ester, prisms, m. p. 167°, and into the ethyl ester, prisms m. p. 140°.

Allomaltol methyl ether, prepared either by the methylation of allomaltol or by the reduction of *5*-methoxy-2-chloromethyl-*4*-pyrone, when heated with ammonia in a closed tube at 100° gives *5*-methoxy-2-methyl-4-pyridone, needles, m. p. 115°, which gives a picrate, needles, m. p. 205–206°. The dimethyl ether of kojic acid, when similarly treated, yields the corresponding pyridine derivative, which distils at 200°/1mm., and on heating with red phosphorus and hydriodic acid gives *4*: *5*-dihydroxy-2-methylpyridine, prisms, decomp. 280°. and crystallising + 1H_2O at low temperatures.

Comenic acid when heated with ammonia under pressure gives comenamic acid, which when treated with phosphorus pentachloride and then reduced with tin and hydrochloric acid gives the same *4*: *5*-dihydroxy-2-methylpyridine. The dihydroxy-picolines obtained thus from both acids gave on methylation the same *5*-methoxy-*1* : 2-dimethyl-*4*-pyridone (+3H_2O), colourless needles, m. p. 98°. This compound is also formed by treating allomaltol methyl ether with methylamine, thus confirming the position of the methyl groups. Comenamic acid on methylation gives similarly *5*-methoxy-*1*-methyl-*4*-pyridone-2-carboxylic acid ($+4H_2O$), needles, m. p. 208°.



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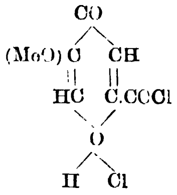

(II)

Sinomenine and Dehydrosinomenine. Parts I and II.

By Kakuji Gotō.

Sinomenine and dehydrosinomenine are alkaloids from the root of a Japanese mountain climbing plant, Sinomenum acutum, Rehd. et Wils., Menispermaceae. The first of them, sinomenine was first isolated by Katsuta Taguchi in a pure crystalline state. He named at first the alkaloid Cocculine (and afterwards Cucoline), according to the obsolute name, Cocculus diversifolius, Diels., formerly given to this plant. He analysed the alkaloid and assigned the formula $C_{17}H_{29}NO_3$ (and afterwards $C_{16}H_{20}NO_3$). Although his descriptions on the melting point, which is 158°, and on the other properties seem to be fairly well established, his formula seems unfortunately to be incorrect. (Tokyo Iji Shinshi, 1919, Dec. 13th.)

Shortly after Taguchi, J. Ishiwari published his pharmacological investigations of the alkaloid, in which some of the chemical properties are also given. (Chugai Iji Shimpo, 1920, No. 959 and 1921, No. 991.)

Succeeding Taguchi, I took up the investigation of the alkaloid and was working several years on it, when in June, 1923 a paper on the same alkaloid was read by Heizaburo Kondo and Eiji Ochiai before the Pharmaceutical Society of Japan (J. Pharm. Soc. Japan, 1923, No. 497, 511). This instigated me to publish my thitherto obtained results in July, 1923, in the Chemical Society of Tokyo (J. Chem. Soc. Japan, Vol. 44, 795, 1923). The results of the last named two authors were in accordance with those of mine in main respects, but in some points they were in advance of me and in others I. Kondo and Ochiai pointed out on the authority of a specialist that the botanical name of the plant must be Sinomenum acutum, and hence the name sinomenine is generally accepted. These two authors published the second report in Dec. 1923 (J. Pharm. Soc. Japan. 1924, No. 503, 8), and I read the second paper in May this year in the Chemical Society of Tokyo. (This report will be published soon in the journal.) Here, for brevity's sake, I wish to resume the results of my investigation, published in these two reports and ask the readers to refer to the originals, as regards the investigations of those two authors.

Isolation.

Finely sliced root of the plant is just covered with 0.5 per cent hydrochloric acid and, after standing about a week at ordinary temperature, it is filtered through cloth. The filtrate is added with concentrated solution of sodium carbonate so long as yet greyish precipitate is formed. This precipitate does not contain alkaloids appreciably, so that it is filtered off quickly. The brown alkaline solution thus obtained is shaken vigorously with chloroform three times. The alkaloids are again extracted from the chloroform solution with 0.5 per cent hydrochloric acid. The latter is concentrated under diminished pressure and on standing the hydrochlorides of sinomenine and dehydrosinomenine crystallise out directly. This process is repeated two or three times and at last the solution is decolourised with animal charcoal. The yield of raw sinomenine hydrochloride is about 0.5 per cent, mixed with a small quantity of the hydrochloride of dehydrosinomenine.

Sinomenine, its properties.

Free base melts at 159–162°. It is easily soluble in alcohol, aceton and chloroform, very little soluble in water and ether and almost insoluble in benzene and ligroin. It forms crystalline salts with hydrochloric, hydrobromic, hydroiodic and nitric acid, but the salts with sulphuric and acetic acid are not crystallisable. The hydrochloride contains two molecules of water of crystallisation and melts at 231° (incorr.). Its solubility in cold water is 4.8 per cent. Elemental analysis gave the following results. $C=69.95$, $H=7.54$, $N=4.33$; $C_{19}H_{23}NO_4$, requires

C=69.38, H=7.04, N=4.25 per cent. Cryoscopic measurement in nitrobenzene gave the molecular weight of 310. Analysis of the hydrochloride (Cl=9.93 per cent), iodomethylate (I=27.41 per cent) and chloroplatinate (Pt=18.81 per cent) gave nearly the same result.

Sinomenine is laevorotatory. Its specific rotatory power in 0.6% chloroform solution is −73°.92 (by sodium light, at 25° C). The hydrochloride is also laevorotatory.

Colour reactions of sinomenine are as follows: with conc. sulphuric acid, not coloured; with conc. nitric acid; reddish yellow; with vanadine sulphuric acid, deep green; with molybdane sulphuric acid, violet, etc.

Sinomenine is precipitated by ordinary alkaloid reagents. All these precipitates are amorphous. The chloroaurate, suspended in water, decomposes after several hours, giving dehydrosinomenine and a brown syrupy matter. Reducing property of sinomenine is remarkable. Thus it precipitates hydrate of manganese dioxide from 0.5 per cent solution of potassium permanganate. Its chloroaurate decomposes by itself on standing and metallic gold is precipitated. From 1 per cent solution of silver nitrate silver is separated on the addition of sinomenine in cold; the solution taking sometimes a reddish violet colour for a moment. Solution of sinomenine hydrochloride produces a violet colour, when added with dilute solution of ferricyanide, made alkaline with sodium carbonate, and under the favourable condition dehydrosinomenine crystallises out directly. This violet colour is taken up by chloroform in much deeper nuance and is observed even in 1 : 100,000th dilution of sinomenine. The colour in chloroform disappears on the addition of an acid or on standing overnight. In all these reactions and also by the action of ferric chloride on the solution of sinomenine hydrochloride dehydrosinomenine is formed with varying yield (see further). But sinomenine neither reduces mercuric acetate nor restores the red colour of decolourized solution of magenta with sulphurous acid.

Diazoreaction of sinomenine is beautiful. An intense red colour is produced when a few drops of 0.5 per cent solution of sinomenine made slightly acidic with hydrochloric acid, are added to an alkaline solution of diazobenzene sulphonic acid. The colour is produced even in one millionth dilution of sinomenine, thus nearly fifty times exceeding that of morphine hydrochloride in sensitiveness. This property may be taken use of in the study of the distribution of sinomenine, when injected in animal bodies.

Properties of Nitrogen and Oxygen Atoms.

Sinomenine is a tertiary base. Its aqueous solution shows a marked alkaline reaction. It was thitherto neither benzoylated, acetylated nor methylated on its nitrogen atom. It does not give Liebermann's nitroso-reaction. On the contrary, the method of Herzig and Meyer reveals the presence of one methyl group attached to the nitrogen atom (4.3%; calculated 4.56%). Monomethylamine was isolated in two cases, once in the distillation of sinomenine with zinc dust, and the other time with baryta.

Iodomethylate of sinomenine (m. p. 255° dec.) is easily formed when sinomenine and methyliodide is mixed in alcohol solution. Recrystallised from water, it contains one molecule of crystal water.

Of the four oxygen atoms contained in sinomenine, two exist as methoxyl (18.27 per cent; $2CH_3O$ require 18.85 per cent), one as carbonyl (oxim, m. p. 233°: N=8.7 per cent; $C_{19}H_{24}N_2O_4$ requires N=8.1 per cent), and the last as hydroxyl.

The existence of hydroxyl was suspected from the facts that a deep green colour is produced by sinomenine on the addition of ferric chloride and that sinomenine is easily soluble in 10 per cent caustic alkali. But it could be neither methylated with dimethylsulphate in a usual way, nor acetylated with acetylchloride or with acetylanhydride. Kondo and Ochiai showed that it could be benzoylated by heating it at 100° C with bezoylanhydride. Following their description, I could confirm the fact, the yield amounting to 30% of the alkaloid used

Benzoylsinomenine (m. p. 225°) gives no ferric chloride reaction. Diazoreaction appears very slowly with it, perhaps the reaction is produced according as benzoylsinomenine is hydrolysed. Determination of benzoyl group by the hydrolysis with caustic soda and titration, gave the number corresponding to one benzoyl group (23.98 per cent; $C_{26}H_{27}NO_5$, requires 23.55 per cent.)

Kondo and Ochiai could moreover prepare monomethylsinomenine by the action of diazomethane on sinomenine, and showed the existence of carbonyl group, independent of the hydroxyl, by converting the monomethylsinomenine into its oxime. I can not yet proceed to this point, but there is now reason to believe that sinomenine can also be methylated by dimethylsulphate to a very small extent. 10 grams sinomenine, methylated with caustic soda and dimethylsulphate, gave 0.5 gram delicate needle, which on the contrary to sinomenine, gave neither ferric chloride reaction nor diazoreaction. It dissolves in concentrated sulphuric or hydrochloric acid with beautiful blue colour, while sinomenine itself dissolves in these reagents almost colourless. Only scarcity of the yield prevented me to proceed further.

Reduction.

Sinomenine seems not to be reduced by common reducing agents such as tin and hydrochloric acid, zinc and acetic acid, or sulphuric acid, and ammonium sulphide. But it is easily hydrogenated by molecular hydrogen, using platinum black or colloidal palladium as catalyst. By the latter method, hydrosinomenine is obtained in 60 per cent yield. The results of analysis show that the substance thus obtained is formed by the addition of two atoms of hydrogen to sinomenine ($C=70.76$, $H=8.19$, $N=4.55\%$; $C_{19}H_{25}NO_4$ requires $C=68.84$, $H=7.6$, $N=4.23$ per cent).

Hydrosinomeine (m. p. 201°) is more soluble in ordinary solvents than sinomenine itself. Colour reactions and precipitation reactions are almost indentical with those of sinomenine. It shows, in particular, the ferric chloride reaction and diazoreaction. The sign of rotation is opposite, i. e. $[\alpha]^{21.5}_{D}=+193^{\circ}.58$ in chloroform and $[\alpha]^{24}_{D}=+33.^{\circ}06$ in weakly acidic solution. No salt of hydrosinomenine is yet obtained in crystalline form. Iodomethylate (m. p. 268° dec.) is prepared as with sinomenine. Hydrosinomenine combines with hydroxylamine (oxime m. p. 211°).

Hydrosinomenine could neither be benzoylated nor acetylated by usual methods. In an attempt to methylate it with dimethylsulphate, a larger part of hydrosinomenine was only recovered from the reaction mixture. Benzoylation with benzoylanhydride, as will be described further with sinomenine, was not successful here.

Attempts to obtain sinomenine from hydrosinomenime by the use of mild oxidising agents, such as dilute solution of pottassium permanganate or of ferricyanide and soda gave no expected result.

Bromination.

Sinomenine takes up four bromine atoms, when acted by the vapour of the latter under a bell-jar. (2 grams of sinomenine takes 2.4 grams of bromine, one bromine atom enters as hydrobromic acid, forming the salt.). From aqueous solution of sinomenine hydrochloride, yellow or brown syrupy matter is precipitated on the addition of bromine, but it was impossible to make it crystallise. As Ishiwari first indicated, bromination is more successful in glacial acetic acid, when one part of bromine is added to two parts of sinomenine hydrochloride, dissolved in the solvent. If bromine is used in a larger quantity, only syrupy matter is obtained. By careful purification, two isomers were unexpectedly isolated from this reaction product. From the content of bromine, the both seem to have the same formula $C_{19}H_{22}NO_4$ Br. The property of the both substances may be conveniently compared as follows.

		Bromsinomenine.	Isobromsinomenine.
Solubility in alcohol.		soluble in cold alcohol.	insoluble in hot alcohol.
M. P. of free bases.		153°	214°
Content of bromine.		19.91 per cent	18.99 per cent.
M. P. of the hydrobromide.		110 (with 3 Aq.). 231 (anhydrous)	229 (anhydrous).
M. P. of Iodomethylate.		80	210–211.
Ferric chloride reaction.		light green	is not produced.
Diazoreaction.		deep red	no colour is produced.
Reduction of gold chloride.		strong	very weak.
With molybdane sulphuric acid.		deep violet	violet at first and quickly pink.
Rotatory power	free base.	$[\alpha]^{25}_{D} = -2^{\circ}.62$	$+14^{\circ}.65$
	hydrochloride.	$[\alpha]^{22.5}_{D} = -30^{\circ}.19$	$+51^{\circ}.79$
Yield.		80 per cent of the total.	20 per cent of the total.

As is seen from the above table, these two isomers are separated by boiling alcohol, in which isocompound is not soluble. Isobromsinomenine shows a remarkable divergence in its property from sinomenine and bromsinomenine, in as much as it gives neither ferric chloride reaction nor diazoreaction and its violet colour in Froehde's reagents turns quickly pink. From these facts it may be inferred that in isobromsinomenine the hydroxyl group might have been replaced by bromine atom. But in that case, the content of bromine must be naturally much higher.

Dehydrosinomenine.

Dehydrosinomenine, which was first prepared by me from sinomenine through mild oxidising agents, was found afterwards occurring actually in the root of the plant. As the hydrochloride of dehydrosinomenine is much less soluble in water (0.6 per cent) than that of sinomenine, it can easily be separated by boiling the crude sinomenine hydrochloride with water. The content of the dehydrosinomenine in the plant seems to be very small. It makes up 2–3 per cent of the crude sinomenine hydrochloride.

The free base of dehydrosinomenine is precipitated from the solution of its hydrochloride by soda in fine rosettes and has m. p. 245°. The hydrochloride does not melt above 280° and the m. p. of the iodomethylate is 261°. Colour reactions of dehydrosinomenine are nearly the same with those of sinomenine, with only a marked difference that it takes yellow colour with diazobenzene sulphonic acid, instead of red. It forms also an oxime, which decomposes at 265°.

Hydrogenation of dehydrosinomenine was carried out in the hope of obtaining sinomenine or hydrosinomenine. This hope was baffled. From 21 grams of dehydrosinomenine hydrochloride, reduced with hydrogen in presence of colloidal palladium, were isolated: 7.5 grams alcohol soluble base (m. p. 245°), 1 gram alcohol insoluble base (m. p. 252°, dec.) and some brown syrup. Hydrosinomenine might have been formed here, but could not be isolated, as it is difficulty crystallisable in presence of syrupy matter, and as no salt of it is known in crystal form. The first base has the same melting point as dehydrosinomenine, so that it may be the latter, which escaped the hydrogenation. But there is some difference in its property, the iodomethylate melts at 277°, and the rotatory power is much higher (free base has $[\alpha]^{25}_{D} = +260°.41$ and the hydrochloride $[\alpha]^{25.5}_{D} = +141°.24$). The solubility of the hydrochloride in water is 12 per cent and that of the iodomethylate is 5 per cent.

Bromination of dehydrosinomenine, which was carried out in the exactly same manner as with sinomenine, gave bromsinomenine (m. p. 153°) only. It may, therefore, be assumed that the action of bromine on sinomenine may be oxidation at first and the dehydrosinomenine thus formed may undergo bromination, or two atoms of bromine are added to dehydrosinomenine and then the elimination of one molecule of hydrobromic acid ensues.

Destructive Oxidation and Fusion with Kali.

Destructive oxidation of sinomenine and its derivatives with potassium permanganate, chromic acid, hydrogen peroxide, nitric acid, bromine, iodine as well as with the excess of silver nitrate, ferric chloride, gold chloride, and alkaline ferricyanide gave no definite results. In most cases, only a brown syrupy matter was obtained. When nitric acid was used, oxalic acid was sometimes isolated. (found, Ag=70.43 per cent; $C_2O_4Ag_2$ requires Ag=70.20. per cent).

Careful fusion of sinomenine with caustic kali at about 200° yielded in 10 per cent yield a white crystal to the ether, after the fused mass was treated with 10 per cent ice-cold sulphuric acid. This substance melts at 169°, thus at the same degree with hemipinic acid, but it shows no property of the latter. It is almost insoluble in hot and cold water, in cold alcohol, and in dilute caustic soda. Concentrated caustic soda dissolves it in a brown solution, but the solution in dilute ammonia shows a lyrac colour, which turns brown on standing. The ferric chloride reaction in alcoholic solution is brown, but it turns purple after the addition of soda. It gives an intense nitrosoreaction. On warming it with concentrated sulphuric acid gently, smell of sulphur dioxide is produced, while no carbonisation is as yet observed.

Decomposition with benzoylanhydride.

Kondo and Ochiai showed that the decomposition of alkaloids of tetrahydroisoquinoline group by heating them with excess of benzoylanhydride, which was so often successfully used by Gadamer and others, could be applied also to sinomenine. They obtained thus from sinomenine a nitrogen free base of m. p. 206°, which shows at least one unsaturation, revealed by the method of Wijs.

I repeated the reaction with the identical results. The new substance contains no nitrogen, it melts at 206° and seems to have the formula $C_{25}H_{20}O_5$ (found, C=75.80, H=6.19; $C_{25}H_{20}O_5$ requires C=75, H=5 per cent). Moreover I could oxidise this substance into an orthoquinone (found, C=69.42, H=4.07, $C_{25}H_{18}O_7$ requires C=69.8, H=4.2 per cent). This quinone is almost cinnober red and melts at 210°. It condences with *o*-phenylenediamine in acetic acid solution, giving yellow needle of m. p. 254°, which is to be regarded as a phenanthrophenazine (found, N=5.50; $C_{31}H_{22}O_5N_2$ requires N=5.58 per cent). This condensed product dissolves with beautiful violet colour in concentrated sulphuric acid, and takes pink colouration on the surface, when poured with concentrated hydrochloric acid.

The best condition to obtain the substance of m. p. 206° is to heat one part of sinomenine with five parts of benzoylanhydride in a strong test-tube, tightly stopped with a rubber stopper, in a glycerine bath at 160–180°, about 6 hours. After cooling, the brown mass is dissolved in chloroform and precipitated with ether. It is purified in a chloroform solution by shaking with dilute hydrochloric acid and then with soda. Yield 35 per cent. It can be recrystallised from boiling acetic acid. It gives neither ferric chloride reaction nor diazoreaction in chloroform solution. It dissolves in concentrated sulphuric acid with brown colour. Fused with kali, it gives much benzoic acid, so that the hydroxyl group of sinomenine is retained here benzoylated. Attempt to obtain oxime of this substance is not yet succeeded. Determination of unsaturation after Wijs gave the value 0.833, thus nearly equal to one double bond. After the titration, I obtained pale yellow crystal from the solution, which is very soluble in ether and shows m. p. 186°. It contains iodine as well as chlorine. The results of analysis are not concordant.

Bromination of the substance of m. p. 206° in chloroform gives hexagonal leaflet, which melts at 228°. Two derminations of bromine gave 21.24 and 21.07 per cent respectively. This would correspond to the formula $C_{18}H_{15}O_4$ Br, which requires Br = 21.33 per cent. Then the benzoyl group might have been hydrolysed in the reaction, which is, however, very improbable. The substance of m. p. 206° was not much attacked in chloroform solution by passing dry hydrochloric acid gas for about thirty minutes.

The substance of m. p. 206° is very resistant against potassium permanganate in acetone solution. It took four days that one gram of the former decolourised 0.8 gram potassium permanganate, even when the mixture was warmed from time to time. From this fact there is some uncertainty about the existence of a vinyl side-chain in the substance of m. p. 206°, for, in that case, it must give a carboxylic acid in the above treatment. This point requires further investigation.

Distillation with Zinc Dust.

Kondo and Ochiai reported that they were successful in obtaining phenanthrene by the distillation or sinomenine with zinc dust. I have not succeeded in this point, although I tried the distillation three times under varying conditions. Once, ten grams of sinomenine hydrochloride were distilled with zinc dust in hydrogen atomosphere, and three drops of yellow liquid were obtained, which soon beautifully crystallised. The crystal became brownish on standing and a greater part of it turned out resinous after some time. Free base of sinomenine was distilled with zinc duct twice, one time in hydrogen and the other time in nitrogen atmosphere. But, in these two cases, minute crystals were recognized in the syrupy matter, but not enough to be isolated.

In every case, monomethylamine was isolated from the dilute hydrochloric acid, which was destined to absorb the basic gaseous products. A brown oil was alse obtained in minute quantity, which smelled after pyridine.

Conclusion.

From the results described above, the formula of sinomenine $C_{19}H_{23}NO_4$ may be resolved as follows.

$$C_{16}H_{13}\left\{\begin{array}{l} O.\,CH_3 \\ O.\,CH_3 \\ OH \\ O\ (\text{carbonyl}) \\ N\text{-}CH_3 \end{array}\right.$$

For the nucleous, Kondo and Ochiai assumes phenanthrene (C_{14}) in its partially hydrated form. This nucleous, combined with the remaining two carbon atoms and one nitrogen atom may constitute a condensed ring system, made of four rings, which contains a grouping somewhat like tetrahydroisoquinoline, just as morphine or apomorphine does.

This conclusion may be correct, for, although I can not yet take out phenanthrene from sinomenine by the distillation with zinc dust, yet the substance of m. p. 206° seems to be a derivative of phenanthrene, giving an orthoquinone, which shows similar character to that of phenanthrene quinone.

The substance of m. p. 169°, obtained from sinomenine by fusion with kali is now under closer investigation. It is hoped that the determination of the constitution of this substance will throw some light on the constitution of sinomenine itself.

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[Received, September, 8th, 1924]

On Vitamin C in the Green Tea.

By MASATARO MIURA, and MICHIYŌ TSUJIMURA.

The authors observed that green tea has a fairly high antiscorbutic potency, while black tea almost lacks this power. This difference may be considered to be caused by the different processes of manufacture of these teas. In the manufacture of green tea the fresh leaves are withered by steaming, and taken in a low wooden flame with paper bottom over the charcoal fire, spreading and drying by rolling over and over for 2–3 hours, so as to reduce its oxidation and to retain the green color as much as possible. But in the manufacture of black tea the cellular tissues of the fresh leaves are first broken mechanically, and exposed to the direct sun light for some hours and then are fermented at a moderately high temperatures, completely converting green colour into brown on account of oxidation in the process.

As the antiscorbutic potency is most easily destroyed by oxidation at high temperature so it is quite natural that the authors found the potency is nearly absent in black tea.

The method used by the authors is followed to the one which is schemed by the member of the Lister Institute of Preventive Medicine, London. Guinea pigs of 270-330 grammes are fed with the mixture of equal volumes of oats and wheat bran ad libitum, and 40–50 c.c. of the milk autoclaved at 120° for an four per day as the basal diet for twelve days, and then the infusion of the tea to be tested is added into the above foods. The tea is always weighed and extracted with a few c.c. of hot water of 60°–70° for 5–7 minutes whenever the impression is required, and to the freshly prepared infusion a few drops of the autoclaved milk mentioned above is added and the mixture is given to the animals by means of forced feeding. The noxious effects of tannin present are modified by the addition of the milk.

Thus the iufusion obtained from one gram of the green tea of last year sold in stores here has been proved completely to prevent the animals from scurvy for more than 60 days, and sometimes no scorbutic sign is shown for 108 days.

It should be kept in mind that the weight of the animals may be decreased when fed with such a substance containing a considerable amount of caffeine and tannin besides vitamin C, even the amount of vitamin C given is increased by a more addition of the substance, as the influence of these intermixtures thus be increased, and also the terms of existence of the animals may be shortened. For this reason, the determination of its antiscorbutic value always requires the *post mortum* examination.

The antiscorbutic potency of the green tea is proved not due to tannin and caffeine, and the experimental results as to the antiscorbutic value of both new and old teas under investigation are shown in the following, provided that all of these contain 4–5% of moisture.

New tea	0.4–0.6 g. per day.
One year stored tea	0.75 „ „ „
Two years „ „	1.00 „ „ „
Four years „ „	negligible „ „

It is also noticed that even an old tea, in case it has a comparatively high lusture and aroma, gives a fairly high potency, and that the potency of the coarse tea (Băn-Chă) though manufactured in this year, is not much significant.

Studies on the Colloidal Substances in Alcoholic Beverages with the Interferometer.

By TEIZŌ TAKAHASHI and HŌBUN ŌMACHI.

The Löwe's interferometer was used for the determination of the colloidal substances in alcoholic beverages, especially in various beers and beer worts by Adler and Luers (Zeit f. d. Ges. Brauw. 1916 Nr. 3–Nr. 6.). No consideration was however given as to the relation existing between the so called "Colloid Number" measured by the interferometer and the analytical percentage of the actual colloidal substances present.

The authors investigated how much an accuracy should be obtained with the instrument using very common colloidal substances such as dextrin and peptone, and tried to find a more suitable substance for removing the colloidal substances than animal charcoal using blood charcoal, barium phosphate etc. for the investigation but these gave no satisfactory result. After all the animal charcoal of the Maerck's brand was found to be the best and most suitable for this purpose.

In the determination of the colloidal substances in the beer, it was diluted with ten volumes of distilled water, 0.5 g of the animal charcoal was added to 50 c.c. of the sample and they noticed that the "Colloid Number" thus obtained is almost equal to (hundred times of) the number of grams of the actual colloids present in 100 c.c. of the sample solution, assuming dextrins and proteins were the main colloidal substances in the beverage.

The following table shows according to the authors' experiments the relation between the "Colloidal Number" and the actual percentage of the colloids present.

Beer	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7
Dextrin	2.07	2.07	2.11	2.77	2.16	1.87	1.89
Protein	0.12	0.126	0.13	0.09	0.13	0.11	0.119
Sum	**2.19**	**2.19**	**2.24**	**2.86**	**2.26**	**1.98**	**2.00**
"Drum Number"	735	760	771	1023	753	749	780
"Colloid Number"	**2.30**	**2.28**	**2.35**	**2.77**	**2.26**	**2.29**	**2.30**

In the case of "Sake," having the same dilution as that in the case of the beer, a less amount of the charcoal was sufficient for the purpose, thus 0.003 g of the charcoal was suitable for "Sake" having a comparatively smaller "drum number" as 1085, while a larger amount of the charcoal, say 0.005 g was required for one which having a larger "drum number" as 1291 or 1331.

JOURNAL OF THE AGRICULTURAL CHEMICAL SOCIETY OF JAPAN.

Vol. 1, No. 2. November, 1924. No. 2.

ABSTRACTS FROM THE ORIGINAL PAPERS.

On the Distribution of Azotobacter in Relation to the Reaction of Soils in Japan.

By Unokichi. Yamagata.

This investigation was made to deal with the distribution of various kinds of Azotobacter in three hundred samples* of soils collected in Japan, and with the relation of their growth to the reaction of soils, especially with the concentration of hydrogen-ion.

Three trypes of Azotobacter; viz., Azotobacter Chroöcoccum, Azotobacter Beijerinckii, and Azotobacter Vinelandii were isolated from one hundred samples and they were cultured in pure state according to the method of the author and Aoi. The distribution of Azotobacter in soils of cultivated. land in Japan is shown in the following table:

Field.	Number of Samples examined.	Number of Samples containing Azotobacter.	Number of Samples containig Azotobacter		
			Chroöcoccum.	Beijerinckii.	Vinelandii.
Paddy.	137	45	25	15	5
Upland.	163	55	41	4	10

*These Samples represent various cultivated soils from the extreme south to the north of Japan.

Districts where samples collected.	Number of Samples examined.	Number of Samples containing Azotobacter.	Percentage of Samples containing Azotobacter.
Hokkaido.	10	1	10
Tohoku.	80	8	10
Hokuriku	10	1	10
Tosan.	20	3	15
Kwanto.	42	17	40
Tokai.	19	7	37
Kinki.	10	4	40
Chugoku.	25	12	48
Shikoku.	18	6	33

Kiushu.	38	24	63
Okinawa.	6	4	66
Taiwan.	22	13	59
Sum	300	100	33

From these results, it is clearly seen that azotobacter is distributed in soils of paddy as well as in upland fields, and also there is a close relation between the distribution and the climatic condition of the districts. The number of percent of soil-samples containing azotobacter to the number of soil-samples examined was only 10 in the northern part of Japan, but more than 60 in the southern part. Azotobacter Chroöcoccum and Azotobacter Vinelandii were found mostly in the soil of upland field, while Azotobacter Beijerinckii in the soil of paddy field.

The distribution of these organisms and the reaction of the soils examined, are as follows:

Reaction of Soil.	Number of Soil-samples examined.	Number of Azotobacter contained in Soils		
		Chroöcoccum.	Beijerinckii.	Vinelandii.
Acid	119	0	0	0
neutral	76	3	11	0
slightly alkaline	62	26	20	0
alkaline	43	37	0	3
Sum	300	66	31	3

Thus, among these three, Azotobacter chroöcoccum was most largely distributed, and especially in the alkaline soils; Azotobacter Beijerinckii was less common and found in nearly neutral soils; and Azotobacter Vinelandii was least common and presented in only the alkaline soils. Azotobacter was always found in the soils containing wood-ash, calcium carbonate, or sea shells.

The properties of the organisms isolated are shown in the table below.

	Chroöcoccum type	Beijerinckii type	Vinelandii type
Form and size.	short plump rod, gen. in pairs $2.3\mu \times 3.4\mu$	large oval $2.3\mu \times 4.7\mu$	oval $1.8\mu \times 3.4\mu$
Motility	motile, in young culture only	no	motile
Coloration on Ashby agar 28 to 30°	A. white B. brownish black	white sulphur-yellow	white greenish yellow; soluble
Coloration in Ashby solution 28 to 30°	A. white, turbid B. brownish black surface film.	white, turbid white, pellicle formation.	greenish yellow yellowish pink

Colony on Ashby agar plate 28 to 30°	round, pasty, dark concentric ring in the center	somewhat round moist, wrinkled surface	round, semitransparent

The quantities of nitrogen fixed by these organisms per one gram of mannit were as follows:

Chroöcoccum type	Bejerinckii type	Vinelandii type
6.28 mg.	4.87 mg.	10.01 mg.

These organisms were cultured in Ashby solution of different P_H, and the optimum P_H and limiting P_H were noted. For the determination of P_H, the electrometric method that was described by Itano and modified later slightly by the same author was used, and the P_H of the solution was determined by titrating electrometrically with $N/10$ HCl and $N/10$ $NaOH$. One cubic centimeter of forty-eight hour old culture in Ashby solution of P_H 7.0 was trasferred into 19 c.c. of the culture solution of respective P_H, so that total volum became 20 c.c. in every case, and the number of bacteria in 20 c.c. medium was counted after 96 hours. Also the rate of growth was noted after 48, 96 and 240 hours, and the final P_H of the medium was determined. The result obtained was as follows:

Azotobacter Chroöcoccum: Better growth took place in media P_H 7.0, and the optimum P_H seemed to lie between 7.45 and 7.60, a marked beneficial effect of $CaCO_3$ on the growth was indicated by the fact that in the control Ashby solution with $CaCO_3$, the organism grew to 484 millions, while the maximum growth was 176 millions in the others.

Azotobacter Beijerinckii: The rate of growth was more markedly influenced according to the different P_H values than in the case of other azotobacter, for instance, at P_H 7.0 a good growth of Azotobacter Beijerinckii took place at the end of forty-eight hours, while at all other reactions the organisms were inhibited, this organism was not influenced by the presence of $CaCO_3$ or at least not so much as others, the optimum P_H seemed to lie between P_H 6.65 to 6.75.

Azotobacter Vinelandii: The behavior of this organism was similar to that of Azotobacter Chroöcoccum except the inhibitory action by P_H below 7 was slightly greater, much greater beneficial effect of $CaCO_3$ was noted; viz., 944 millions in the control and 72 millions in the medium of original P_H 8.6 and of final P_H 7.0.

Further, the acidity of soils was defermined by the method of Christensen comparing with the method of Itano's electrometric method, and the fact that the reaction of soils containing azotobacter was alkaline in most cases and that no azotobacter was found in the soil of acid reaction was observed. The following table shows the relation between the reaction of soils and the distribution of Azotobacter:

Reaction	P_H	Number of Soils examined	Number of Soils containing Azotobacter	Azotobacter		
				Chroŏcoccum	Beijerinckii	Vinelandii
acid	less than 5.9	29	0	0	0	0
weak acid slightly acid	6.0-6.4	90	0	0	0	0
neutral slightly alkaline	6.5-7.2	76	8	3	5	0
weak alkaline	7.3-7.7	62	52	26	14	12
alkaline	more than 7.8	43	40	37	0	3

Summary.

1. The distribution of Azotobacter in various cultivated soils in Japan was examined and it was found that this organism was present more in the soils of warmer climate region. The precentage of this soils containing azotobacter all was about 30.
2. Azotobacter was found in the soils containing lime, wood-ash or sea-shells, and also in most soils of alkaline reaction (P_H=7.3−7.8) but no azotobacter in the soils of acid reaction (P_H=less than 6.4).
3. Three types of Azotobacter; viz., A. Chroöcoccum, A. Beijerinckii and A. Vinelandii were isolated from the sample soils.
4. The quantities of nitrogen fixed by these organisms are as follows:

	N fixed per gram of mammit.
A. Chroöcoccum.	6.28 mg.
A. Beijerinckii.	4.87 mg.
A. Vinelandii.	11.01 mg.

5. Two types of Azotobacter chroöcoccum and Azotobacter vinelandii were found mostly in the soils of weak alkaline or of alkaline reaction (P_H=7.3-7.8), while the Azotobacter Beijerinckii. in the soils of neutral, or in weak alkaline reaction (P_H=6.5-7.7).
6. Azotobacter was isolated from the soils of paddy as well as from that of upland field. Two types of A. chroöcoccum and A. vinelandii were found mostly in upland soils, while A. Beijerinckii in paddy soils.

7. A pure culture of Azotobacter has easily made according to the method of the author and Aoi.
8. The author has used the apparatus schemed by Itano for determining hydrogen-ion concentration.
9. For the purpose to compare the rate of growth of these organisms in culture media of different P_H, Ashby solutions of different P_H were prepared by electometrical titration.
10. The behavior of Azotobacter chroöcoccum was similar to that of Azotobacter vinelandii, and they are not influenced by alkaline reaction, but the latter was more influenced by acid reaction than the former. Azotobacter Beijerinckii was more greatly influenced at different P_H values than in the case of other two organisms.
11. The optimum and limiting values of P_H for the growth of these organisms were as follows:

Types	Optimum value	Limiting value
A. Chroöcoccum.	7.6–8.3	5.8
A. Beijerinckii.	6.8–7.4	5.8
A. Vinelandii.	7.6–8.3	5.9

12. The beneficial effect of calcium carbonate was noted for the growth of Azotobacter Chroöcoccum and Azotobacter Vinelandii, especially for the latter. Azotobacter Beijerinckii was not so much influenced by this alkaline substance.

Finally, the author desires to express his sincere thanks to Prof. K. Aso and Dr. Itano for their kind suggestions, and also to Dr. S. Saito and C. Matsuoka for their valuable assistance during this investigation.

On the Occurrence of a New Sulphur Compound in Yeast.

by UMETARŌ SUZUKI, SATORU ŌDAKE and TAKAJIRO MORI.

Ten years ago, one of the authors (U. Suzuki) isolated a basic compound from the alcoholic extract of yeast and gave the empirical formula $C_9H_{14}N_4O_4$ to it. It was a colourless prism, melting at 208° (not corr). The aqueous solution was nearly neutral to litmus, and possessed a slightly bitter taste. It was precipitated with phosphotungstic or phosphomolybdic acid, but not with basic lead acetate. It formed also the crystalline picrate, melting at 183°. (not corr.) (Jour. Tokio Chem. Soc., Vol. 34, p.p. 1134–1135. June. 1914)

Further studies on this substance were interrupted for a long time; however, quite recently it was found by the authors that it contains sulphur, consequently the proposed formula must be abandoned. For such a reason the authors prepared this compound again in a large quantity and carefully investigated its chemical nature. The analysis of the free base as well as that of its picrate have shown that the empirical formula should be $C_{11}H_{15}N_5SO_3$.

The isolation of this compound was carried out as follows:—

The alcoholic extract of yeast was evaporated in vacuum. The resulting syrup was dissolved in a little water, and a concentrated tannin solution was added to it. The precipitate thus formed was rubbed with baryta water and filtered. The filtrate was freed from an excess of baryta, and evaporated to a syrup again, and treated with 50 % aceton, whereby a portion remained undissolved. The dissolved portion was evaporated to a thick brown syrup which possessed strong antineuritic properties. After long standing, the sulphur compound separated out from this syrup in colourless crystals, which was recrystallsed from the water solution.

When this base is boiled with dilute acid it is easily hydrolysed, thereby adenine ($C_5H_5N_5$) and a thio-sugar being formed. Although the authors have not yet succeeded to isolate the free thio-sugar in crystalline state, but it was isolated as a nice crystalline phenylosazon, having the melting point of 158–159°, and the analysis of the latter compound agreed with the formula $C_{18}H_{22}N_4SO_2$, that is, phenylosazon of a thio-sugar having the formula $C_6H_{12}SO_4$.

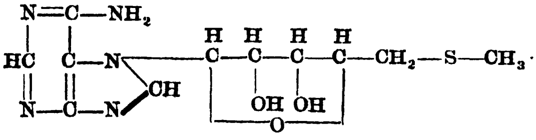
The free base gives the pentose reaction with Bial's reagent, and it gives also furfurol reaction when it is boiled with hydrochloric or sulphuric acid. By evaporating the base with strong nitric acid, the crystals of hypoxanthin nitrate were obtained, which was formed from adenine; the oxidation proceeded more energetically when a trace of vanadium oxide was added to the nitric acid solution, and besides hypoxanthin, crystals of xanthin nitrate were obtained. By shaking the mother liquid of hypoxanthin or xanthin with ether, and after evaporating the ethereal solution, colourless prisms of oxalic acid were obtained. Furthermore, in the remaining aqueous solution the presence of some sulphuric acid could be detected, this shows that a part of sulphur present in the base was oxidized to sulphuric acid.

When it is boiled with dilute hydrochloric acid, and barium chloride is added to it, no precipitate is formed, this indicates that the sulphur present does not exist in the sulphate form. In a cold dilute alkaline solution it gives no reaction with sodium nitroprusside, but it gives a dark red colour when it is previously boiled with concentrated alkali before adding sodium nitroprusside.

When this base is boiled with dilute hydrochloric acid, adding a piece of metallic zinc, there develops a gas having a strong pungent odour, which gives a white turbidity when it is introduced into an aqueous solution of mercuric nitrate or mercuric chloride. As neither the presence of hydrogen sulphide nor mustard oil could be detected in this case, it is most probable that this gas chiefly consists of mercaptane or dimethyl sulphide.

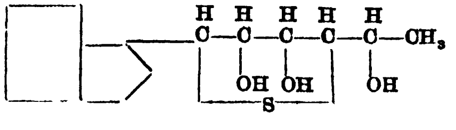
From these observations, the authors concluded that the basic substance is a compound of adenine and a thio-sugar (probably thio-methyl pentose) having one of the following formulae :—

(I)



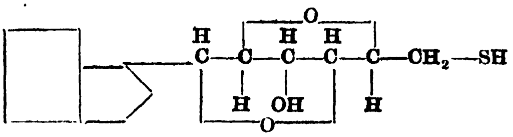
$=C_{11}H_{15}N_5SO_3$

(II)



$=C_{11}H_{15}N_5SO_3$

(III)



$=C_{11}H_{13}N_5SO_3$

Of the formulae above given, the formula (I) seems to agree best with various facts. Further studies will be required to decide whether this assumption is correct. Not only the occurrence of thio-sugar in organisms is quite new, but its combination with adenine is of special interest from the biochemical point of view, because it has a great resemblance with adenosine, a compound of adenine with ribose, which forms an essential part of nucleic acid.

Among the sulphur compounds in animal organisms cystin, cystein, taurin, and taurocholic acids are most popular ones. Further, chondroitic acid forms an essential part of cartilages. The 'etherial sulphates of urine are to be considered as the end product of sulphur metabolism. Mustard oil groups are widely distributed in plant. Recently, a sulphur derivative of histidine betaine, i. e. ergothionin was isolated by Tanret from ergot. J. H. Müller has reported a new amino acid containing sulphur, which is probably ethyl ester of cystein. $C_2H_5S\text{-}CH_2CH\text{-}NH_2\text{-}COOH$. (J. H. Müller: J. Biol, Chem. **56.** No. 1, pp. 157–169). The brain substance of animals contains also not inconsiderable sulphur, but nothing is known about its chemical nature, though it is generally believed that it plays just so important rôle like phosphoric acid in the nervous tissues. The authors suppose that the sulphur compound discovered by them has also a certain function in the yeast cells.

It may also be mentioned here that the alcoholic extract of rice polishings after long standing, always produces an amorphous brown precipitate. B. Suzuki has recently investigated this precipitate, and found that it has a strong stimulating action upon the growth of yeast, though it has no antineuritic action on pigeons (J. Chem. Soc. Japan, 1924). Now it was found by the authors that this precipitate, after being carefully purified, contains a little S. The crude oryzanin prepared from the alcoholic extract of yeast by the phosphotungstic acid method contained also more than 1 % of sulphur. Though it has no direct relation with the antineuritic properties, yet it is very interesting that it is always present in oryzanin preparation of yeast and rice polishings. The question that whether the sulphur compound of rice polishings is same with that of yeast or not still remains to be settled.

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ABSTRACTS FROM THE ORIGINAL PAPERS.

On the Buffer Action of Saké.

by HIDEO KATAGIRI.

1. The acidity of Saké, estimated by the ordinary titration method varies depending upon the qualities and ages etc. from 0.12 % to 0.21 %. But when its P_H value was measured electrometrically, the difference between the maximum and minimum acidity was found to be far smaller than it is to be expected from the titration value. Thus according to the author's experiment, the P_H values of different samples of Saké collected from various districts of Japan, by the colorimetric method were from 3.7 to 3.9., while those by the electrometrical method, from 4.0 to 4.25. Even when Saké was diluted with water, two to five times its volume, the value remained nearly unaltered, still being between 4.0 and 4.25.

This phenomena were considered by the author was due to the high buffer action of the Saké solution. Consequently he investigated to find out, what constituents are exerting this action.

2. When Koji extract was fermented with Saké yeast, an increase in buffer action was always observed, thus showing that certain products possessing higher buffer action were formed durring the fermentation process.

3. Saké was diluted with water from two to five times its volume, and the P_H values were determined either colorimetrically or electrometrically, then a measured quantity of decinormal hydrochloric acid or caustic soda solution was added in successively with an increasing quantity, and thus the P_H values were again determined after each addition, and the results obtained were shown as electro titration curves.

4. In the next experiments, each one of the chief constituents of Saké (*i.e.* alcohol, glucose, dextrin, glycerine, glycocoll, tysosine, peptone, phosphates and

succinic acid etc.) was added to the diluted Saké solution, so as the concentration of that constituent in it become equal to that concentration in the original Saké solution, and the P_H values were determined before and after addition of either hydrochloric acid or alkali and the results were also given as titration curves. Any substance which has a greater buffer action must show a less deviation in the P_H values before and after the addition of either acid or alkali to it.

Comparing in this way the buffer action of the above mentioned constituents the author came to the following conclusions:—

a) The addition of alcohol, glucose, dextrin or glycerine had no effect upon the buffer action and the titration curves before and after the addition of these substances remained nearly unaltered.

b) Peptone, in such a low concentration as it presents in the original Saké solution, exerted no action.

c) Glycocoll, more or less acted as a buffer against acid.

d) Tysosine, though present in a minute quantity, exhibited a distinct action of the buffer toward alkali.

e) Phosphates had only a slight effect upon alkali.

f) A most pronounced action was shown by succinic acid, and the addition of this acid only was enough to increase the strength of the buffer action as much as that in the original Saké solution.

After all, succinic acid played the most important rôle on the buffering action of Saké.

The author further discussed the relation between the sour taste given by Saké, and its P_H value and concluded that the strength of the sour taste is not always proportional to its P_H value, but certain unknown factors should be connected to it.

In the last chapter, the author has given a precise description of his experimental methods.

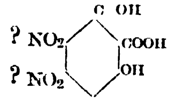
On the Chemical Constitution of β-Acid Obtained by the Hydrolysis of Suzuki's Crude Oryzanin.

By YOSHIKAZU SAHASHI.

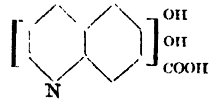
In 1912 Prof. U. Suzuki isolated two crystalline compounds from his crude oryzanin by boiling it with dilute acids, and he provisionally named them α- and β-acids, respectively. The crude oryzanin was prepared from the alcoholic extracts of rice polishings, precipitating the extracts with phosphotungstic acid and then decomposing the precipitate with baryta in the usual way. When this crude oryzanin was boiled with 3% hydrochloric acid for about 2 hours, a brown resinous substance was formed on the surface of the liquid, which was filtered off while hot; and yellowish brown crystals that were formed on cooling the filtrate were collected, washed with water and recrystallized from hot alcohol. By fractional crystallization of the yellowish brown crystals, two kinds of crystals, i. e. α- and β-acids were obtained. They had an acid reaction and were difficultly soluble in ether, in cold water, and in dilute acids, but were somewhat soluble in hot alcohol, and readily soluble in dilute alkali. They gave an intense reaction with diazobenzene sulphonic acid, and also a strong Millon's reaction. The empirical formulae $C_{18}H_{16}N_2O_9$ and $C_{10}H_8NO_4$ were given to α- and β-acids, respectively; but his further investigations on the acids were interrupted, chiefly owing to the difficulty in preparing the material enough from crude oryzanin.

When the alcoholic extracts of rice polishings are dissolved in 20% alcohol and the solution allowed to stand for several weeks, a brown amorphous precipitate gradually settles down at the bottom. B. Suzuki has recently investigated this precipitate and found that a fair amount of β-acid would be formed, together with a brown resinous substance, by boiling this precipitate with dilute acids. The yield of the acid thus obtained was much better than that from crude oryzanin. (Jour. Chem. Soc. Japan, 1924). Consequently it has become much easier for the author to prepare a sufficient quantity of the acid by this method for his investigation. Many kilograms of the brown amorphous precipitate were supplied to the author from the Sankyo & Co, Tokyo, where oryzanin is manufactured in a large scale under the supervision of Prof. U. Suzuki, and thus the author was able to obtain more than 300 grams of β-acid in pure state, which was exactly identical with that obtained from crude oryzanin by Prof. Suzuki.

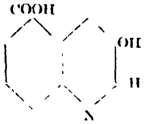
The author found that β-acid has one molecule of water of crystallization, which could hardly be removed at 100° C, but it was quite free from water when dried at 150–160°. According to the results of the anhydrous sample it seemed to the author that $C_{10}H_7NO_4$ would be more suitable for the formula of β-acid than $C_{10}H_8NO_4$, (that was) proposed by Prof. U. Suzuki. He dried his sample at 100° for analysis, therefore, it was probably not quite free from water. The author has prepared various compounds of this acid, i. e. $C_{10}H_6NO_4{\cdot}Na$, $C_{10}H_6NO_4{\cdot}K$, $(C_{10}H_6NO_4)_2Cu$, $(C_{10}H_6NO_4)_2Ba$, and Ag-, Ca-, NH_4-salts, and also $C_{10}H_6NO_4{\cdot}CH_3$, $C_{10}H_6NO_4{\cdot}C_2H_5$, $C_{10}H_4NO_4(CO{\cdot}C_6H_5)_3$, etc. The formation of these salts and esters, proved it to be a monocarboxylic acid; while that of the benzoyl derivative confirmed the presence of two hydroxyl groups. Thus the formulae may be written as $C_9H_4N(OH)_2COOH$.

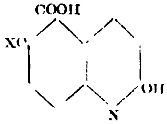
When the β acid was treated with strong nitric acid, a dinitro-compound like juglonic acid 

, besides a fair amount of oxalic acid, was formed. Finally, quinoline was obtained by zinc dust distillation of the β-acid, and this was identified by its picrate.

The author has thus concluded that β-acid is probably dihydroxy-quinoline carboxylic acid 

The relative position of $(OH)_2$ and COOH groups remains still under his investigation; but from the evidence of the formation of the dinitro-compound and oxalic acid, the author thinks that the β-acid will very likely have either one of the following formulae:





A New Method of the Determination of the Water in Green Tea.

By TOKITAKA SHIBA.

A cobalt chloride test-paper was prepared for the purpose of determining the water in green tea. Its preparation and application are very simple, and it can be used even by those who have no special knowledge of chemistry. This test-paper changes the colour shades according to the amount of the water present in the tea. The tea which does not change the blue colour of this test-paper No. 30 is suitable for preservation, that which changes (it) into red will change in its property, and the one which gives a violet colour can be kept only for a short period without changing its property. The test-paper can also be used in the quantitative determination of the water in tea, and the difference between the value obtained by the usual gravimetric method and that by the above method is about ± 0.3 per cent. With this test-paper the content of water can be determined in tea in any amount and in any vessel.

The sample of the standard of colour used does not last long, and so requires revision from time to time. The use of a table of the shades of colour painted with dye-stuffs is very convenient for a longer period of colour measurements, but it also requires the revision after some time.

The fact that this test-paper can be employed in determining the humidity of a room, where the tea is handled may be of very useful.

The method is, in the opinion of to the author, very profitable to prevent tea from the damage in its manfacture, caused by insufficient drying, which is easily avoided by its application.

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Vol. 1, No. 4 January, 1925. No. 4

ABSTRACTS FROM THE ORIGINAL PAPERS.

The Chemical and Mineralogical Investigations on the Infertile Volcanogenous Soils of the Southern Part of the Prov. Shinano.

By TOYOTARŌ SEKI.

The peculiar infertile soils so called "*misotsuchi*" extending at the surface and some centimetres below the surface in the higher parts of the Tenryugawa Valley (T. V.) and at the southern skirt of the Yatsugatake Volcanoe (Y. S.) were investigated in the laboratory of Central Agricultural Experiment Station and the brief summary will be given here.

The soils form the upmost parts of the old quarternary formation covering the thick strata consisting of the fluvial gravels.

Their inner structures and adjacent topographies suggest that the soils are the *lacustrine* deposits of the later diluvial age. From the modes of occurence and the results of microscopical observations it is clear that they were originally the fine porous *pumiceous volcanic ejectaments* of the andesitic character, which have undergone the decomposition at first under the shallow waters and then at the land surface.

The soils naturally wet exhibit the light reddish brown colours with somewhat spongy structures. The water held by them cannot easily be removed by exposing them to the air in open place in the shadow. When air-dried, they exhibit the faint brownish yellow colours becoming the light porous and fragile fragments which give readily the fine powders on rubbing. If the half-dried fragments are stirred in the water they give the thick turbid liquids which remain almost unchanged for few days showing the high grades of *colloidality*, which give rise to the strong retentive powers of the soils for water.

The air-dried and shifted samples were treated with boiling 20 per cent hydrochloric acid and the substances decomposed by the acid were determined quantitatively.

The results of analyses are as follows :-

	Subs. dec. by HCl	Residue undec.	Water above 100°	Water below 100°	Total Sum
The Soil of Fujimi (Y. S.)	36.72	23.99	11.71	27.72	100.14
The Soil of Ijima (T. V.)	42.68	7.53	13.43	36.26	99.90

The substances decomposed by the hydrochloric acid are constituted of :-

	SiO_2	Al_2O_3	Fe_2O_3	MgO	CaO	Na_2O	K_2O	P_2O_5
The Soil of Fujimi	14.43	19.36	1.97	0.05	0.04	0.16	0.12	0.08
The Soil of Ijima	17.21	22.23	2.20	0.05	0.10	0.17	0.05	0.08

The minor ingredients were omitted here.

The large amounts of the hygroscopic moisture and the substances decomposed by hydrochloric acid and the great deficiencies in lime, potash and phosphoric acid must be carefully noticed. The molecular ratios of the most important ingredients to alumina calculated for our samples and some other soils are given in the following table :-

	Volc Soil of Fujimi		Volc Soil of Ijima		Acid Soil of Mino		Volc Soil of Tokyo	
SiO_2	1.26		1.31		1.20		1.63	
Al_2O_3	1.00		1.00		1.00		1.00	
Fe_2O_3	0.07		0.07		0.26		0.36	
MgO	0.01	0.03	0.01	0.03	0.09	0.14	0.28	0.36
CaO	0.00		0.01		0.01		0.04	
Na_2O	0.01		0.01		0.02		0.02	
K_2O	0.01		0.00		0.02		0.02	
P_2O_5	0.00		0.00		0.01		0.00	

The molecular ratios of the total alkaline bases to alumina in our samples are far less than that in the infertile acid soil of the Prov. Mino and represent the minimum value among the numerous volcanogenous soils hitherto investigated by the author. The molecular ratios of silica to alumina in our soils approach to those in the *lateritic* soils.

The both soils are stained vividly red by acid fuchsin and light bluish green by methylene blue, that is they are distinctly *oxyphilous* and *basophilous* (amphophilous). The facts explain that the soils contain both positive colloids (oxyphilous) and negative colloids (basophilous). The two dyes absorbed by the soils were also colorimetrically determined. The quantities of alumina extracted almost free from silica with 10 per cent sodium carbonate solution by the anthor's method amount to about 3 per cent. The last fact together with the distinct oxyphilous character prove that the soils contain *free alumina* in the form of colloidal hydroxide. The ferric oxide in the soils will mostly be in the form of limonite which is almost indifferent to the aniline dyes. From these reasons the author assumes that the main puts of our samples are constituted of the large quantities of *colloidal clays*, smaller quantities of active *colloidal aluminium hydrate* and less quantities of innert ferric oxide (*limonite*). The presence of free aluminium hydrate shows that the soils are proceeding a step into the *lateritic phase* of weathering, as is suggested by the low values of the molecular ratios SiO_2 Al_2O_3.

The two soils do not exhibit the acid reaction to test paper notwithstanding they are very rich in the colloidal clays and exceedingly poor in alkaline bases. The fact can be explained by the presence of the active gels of aluminium hydrate which "*compensate*" the acid reaction of colloidal clays, as was demonstrated by the author's preliminary experiments. The soils exert — very *high absorptive powers* for ammonia and phosphoric acid and these facts can also be explained by the presence of colloidal clays and aluminium hydrate. In such soils ammonia, potash and phosphoric acid given as fertilizers in ordinary doses are liable to be converted into the unavailable forms.

The unproductiveness of our soils due chiefly to the defect in physical properties and the lack of mineral nutrients. The improvements will be accomplished (1) by the liming and deep cultivation in order to coagulate the colloidal substances and produce the granular structures, (2) by the rich supply of superphosplate and potash salts and the adequate addition of nitrogenous manures, (3) by the propagation of reguminous green-manure plants in order to enrich the soils with humus and organic nitrogen. The practical applications of those methods must carefully be verified by preliminary field experiments.

On the Chemical Constitution of the Cocoon Silk and the Sericin of the Cocoons of Antheraea Yamamai.

By RYUGO INOUYE and MASARU HIRASAWA.

I. The Chemical Constitution of the Cocoon Silk.

(a) The General Composition.

The cocoons were got at Ariake-mura, Minamiazumi-gun, Nagano-ken, and analyzed with the following results, after having get rid of the impurities.

Water	10.77	%
Total nitrogen	16.51	〃
Ash	4.15—5.00	〃

Lime is a predominant constituent in the ash and exists mostly in the form of oxalate in the cocoons.

The various forms of nitrogen in the hydrolysate of the cocoon silk with concentrated hydrochloric acid, were determined as follows :-

	In 100 g of the anhydrous cocoon silk.
Total nitrogen	16.51 g
Nitrogen dissolved in conc HCl	16 37
Nitrogen precipitated by phosphotungstic acid	3.07
Nitrogen not precipitated by phosphotungstic acid	12.82
Nitrogen in the form of ammonia	0 22
Nitrogen in the melanine resulted by hydrolysis	0.18
In the nitrogen precipitated by phosphotungstic acid,	
Arginine nitrogen	0.10
Histidin nitrogen	0 18
Lysine nitrogen	1.14

(b) Total Hydrolysis.

200 grms. of the cocoon silks were hydolyzed with 2500 c.c. of concentrated hydrochloric acid. After 12 hours' boiling the hydrolysate ceased to show biuret reaction, and then amino-acids were separated by the ester method with the following results.

Amino acid	In 100 grms of anhydrous cocoon silk In grm.
Glycocoll	17.83
Alanine	20.16
Leucine	1.23
Aspartic acid	0.26
Glutamic acid	Trace
Serine	2.83
Proline	0.21
phenylalanine	0.22
Tyrosine	5.34

Tyrosine was separated by another way as follows:- 50 g of the cocoon silk were hydrolyzed with 30% sulphuric acid for 12 hours until the hydrolysate has not shown biuret reaction. Then the sulphuric acid was exactly removed by concentrated solution of baryta. The precipitate of barium sulphate thus produced was filtered, and so often washed until the washing did not react with the Millon's reagent. The filtrate and washings were united together and decolourized with animal chacoal. And the solution thus purified was evaporated until tyrosine was crystallized out. It was filtered after standing for 24 hours. The filtrate of tyrosine was evaporated, and the remaining tyrosine was obtained.

These amino acids thus separated were determined by analysis.

II. The Chemical Constitution of the Sericin of the Cocoons.

(a) The Separation of Sericin.

10000 cocoons of Yamamai-moths were cut in two and the chrysalis, the skins casted, and other impurities were removed. The cocoons thus purified, were digested with 40% alcohol in an autoclave under 0.5 atmospheric pressure. The green pigment of the cocoons was dissolved in the alcohol. The cocoons were treated twice in the same way, and thus decolourized nearly in white. Then they were digested again under 1 atmospheric presure for 30 minutes with steam, and filtered through a Buchner-funnel by means of sucking down by a vacuum produced by a filter pump. The filtrate was evaporated into a syrup after having been filtered once more, and dried up on concentrated sulphuric acid. After completely drying, the sericin was ground into powder. The yield of the sericin was 200 grams from 5000 grams of cocoon silk. The sericin thus obtained, contained 3.12% water, 16.85% the total nitrngen, and 1.00% ash.

200 grams of the sericin were hydrolyzed by 2000 c.c. of 30% sulphuric acid. After 78 hours' boiling the hydolysate ceased toshow biuret reaction. The sericin was completely dissolved without any residue. Then tyrosine was at first separated by the same method described as before. From the filtrate of tyrosine other amino acids were separated by the usual method. The amino acids thus obtained were determined by analysis.

The results of the total hydrolysis were as follows :–

Amino acids	In 100 grams of anhydrous sericin
Glycocoll	3.45 g
Alanine	3.99
Leucine	1.50
Sarine	4.38
Aspartic acid	2.96
Glutamic acid	presence
Phenylalanine	2.04
Tyrosine	4.33
Proline	presence

III. Conclusion.

By cmparing the chemical constitution of the Yamamai cocoon silk to those of the tussah produced at the same district, and of "Kinjo–matamukashi" cocoon silk (one of Japanese domesticated slik–worm), the following table is obtained.

Amino acids	Yamamai–cocoon silk.	Tussah–cocoon silk.	Kinjo-matamukashi cocoon silk.
Glycocoll	27.83 %	12.34 %	29.39 %

Alanine	20.16	15.27	16.72
Leucine	1.23	0.27	1.47
Aspartic acid	0.26	2.37	0.03
Glutamic acid	+	+	0.023
Serine	2.83	0.55	3.01
Proline	0.21	0.26	1.106
Tyrosine	5.34	6.62	4.72
Phenylalanine	0.22	0.37	0.64

From the above results glycocoll, alanine and tyrosine are predominant amino acids in the Yamamai silk, as general in the silk, and it stands nearer to the true silk in the chemical composition, than the tussah silk. But that more aspartic acid is contained, when compared to the true silk, and alanine is more in quantity than glycocoll, is similar to the tussah silk. From those facts the Yamamai-silk may be said to stand in the middle point between the true and tussah silk in the chemical composition.

The chemical composition of the sericin of the Yamamai-silk is compared to those of the other sericins, which have been investigated until to-day, in the following table :-

Amino acids	Sericin from Yamamai silk	Sericin from Daiwanishiki silk	Sericin from Italian silk	Sericin from Canton silk	Sericin from the European silk
Glycocoll	3.45 %	3.93 %	1.5 %	1.2 %	0.1 0.2 %
Alanine	3.99	3.53	9.8	9.2	5.0
Leucine	1.50	0.4	4.8	5.8	—
Aspartic acid	2.96	3.91	2.8	2.5	—
Glutamic acid	+	3.0	1.8	2.0	—
Serine	4.38	5.99	5.4	5.8	6.6
Proline	+	0.35	3.0	2.5	—
Tyrosine	4.33	3.27	1.0	2.3	5.0
Phenylalanin	2.04	0.49	0.3	0.6	—

From the above results the sericin of the Yamamai-silk is very much near to that of the true silk in the chemical composition. If it is the fact, the cocoons of the Yamamai-moths must be so easily reeled as those of the domesticated ones, but in practice it is not the case. The reason is probably due to that the calcium salts, cotained comparatively more in quantity on the cocoons, prevent the sericin from dissolving, when the cocoons are boiled and the silk is reeled.

Ueber die Enzymatische Spaltung der Laevulinsäure (Ein Beitrag zur Wirkung der Carboxylase).

VON KANROKU KURONO, TÔSHI FUKAI und SEIJUN TATENO.

Nach C. Neuberg werden α-Ketosäuren wie Brenztraubensäure und Oxalessigsäure durch Einwirkung von Carboxylase in Kohlensäure und Azetaldehyd nach folgendem Schema gespalten.

$$CH_3{\cdot}CO{\cdot}COOH = CO_2 + CH_3{\cdot}COH.$$

$$COOH{\cdot}CH_2{\cdot}CO{\cdot}COOH = 2CO_2 + CH_3{\cdot}COH$$

Andere α–Ketosäuren wie α–Ketobuttersäure, α–Ketocapronsäure, Oxybrenztraubensäure, α–Ketoglutarsäure, Phenylglyoxalsäure, Phenylbrenztraubensäure und Oxyphenylbrenztraubensäure verhalten sich gegen Carboxylase genau in derselben Weise und bilden sich dabei Kohlensäure und die entsprechenden Aldehyde.

In Oxalessigsäure und α–Ketoglutarsäure steht eine der zwei COOH Gruppen gegen CO Gruppe in β–bzw. in γ–Stelle: trotzdem werden die beiden Carboxylgruppen gleichzeitig angegriffen. So kann man vermuten, dass β–oder γ–ketosäuren ebenfalls durch Carboxylase abgespalten werden. Um diese Frage zu entscheiden, haben die Verfasser mit Laevulinsäure (γ–Ketosäure) einige Versuche angestellt und gefunden, dass es tatsächlich der Fall war.

In einem Versuche wurden 20 ccm. 0.5% iger Laevulinsäurelösung, die zuvor mit einer K_2HPO_4–pfufferlösung neutralisiert war, in einen Einhorn'schen Gärkolben gefüllt, mit 2g Trockenhefe versetzt und unter Zusatz von 0.1 % Thymol im Wärmekasten bei 25-30° stehen gelassen. Nach 48 Stunden beobachtete man, das 27% der zugesetzten Laevulinsaure zerlegt wurden, d. h. 62% der in Lösung vorhandenen freien Säure.

Es wurde erfahren, dass der optimum PH–Wert der Lösung für die Carboxylase –Wirkung 4.5% und die optimale Konzentration der Laevulinsäure 0.5%war. 1% ige Lösung wirkte schon schädlich. Ferner wurde beobabachtet, dass das Kalium oder Natriumsalz der Laevulinsäure nicht gespalten war.

Die Verfasser haben später mit grösserer Menge Laevulinsäure gearbeitet und als Spaltungsprodukte derselben Methyl–ethyl-keton isoliert und es als krystallinisches Semicarbazon identifiziert. So kann man den Reaktionsverlauf sich in folgender Weise vorstellen :–

$$CH_3{\cdot}CO{\cdot}CH_2{\cdot}CH_2{\cdot}COOH = CO_2 + CH_3{\cdot}CO{\cdot}CH_2{\cdot}CH_3$$

Laevulinsäure Methyl–ethyl–keton.

So bietet es ein neues Beispiel für die Wirkung der Carboxylase dar. Die Verfasser beabsichtigen die versuche mit verschiedenen β–oder γ–Ketosäuren weiter fortzusetzen. Ferner bleibt die Frage, ob es sich beim Abbau der α-und γ-Ketosäuren um eine und dieselbe Carboxylase handelt, noch zu entscheiden.

A New Method for the Quantitative Determination of Amylo-Liquefying Enzyme.

By Kōkichi Ōshima and Shinichi Itaya.

Make 450 c.c. of starch paste with 7.5 grams (as dry matter) of purified potato starch. 18 c.c. of this paste + 2 c.c. regulating mixtures of M/6 citric acid and M/6 Na_2PO_4 to keep certain hydrogen ion concentration, and digest with 2 c.c. of enzymic solution for 30 minutes at 40°C. Then add 2 c.c. of N/2 NaOH to stop the digestion. Add again 2 c.c. of M/6 citric acid and M/6 Na_2HPO_4, which makes it's total 2 c.c. M/6 citric acid and 2 c.c. Na_2HPO_4. Determine the viscosity of digested liquid at 18°C with Ostwald's viscosimeter of 8 c.c. capacity in which distilled water flows down with 15.0 seconds. With this calculate the enzyme strength from the table shown below.

This table was made experimetally with different concentrations of an enzymic preparation from Aspergillus oryzae. When 1% solution of an enzymic sample digests 1.5% starch paste at 40°C for 30 minutes and the viscosity is 47 seconds (which is same viscosity with 1% paste) then the amyloliquefying activity is 10.

Table:- Activity Scale of Amylo-liquefying Enzyme.

Seconds of viscosity	Amylo-liquefying activity × Y	Seconds of viscosity	Amyloliquefying activity × Y
×20	100.0	×38	16.6
21	87.5	40	15.5
22	75.0	×43	12.5
23	62.5	45	11.2
×24	50.0	×47	10.0
25	48.0	×50	8.3
26	46.1	×53	6.3
×27	40.0	×55	5.0
×28	33.3	57	4.8
29	28.0	×60	3.1
30	27.0	×62	2.5
31	26.0	×65	1.6
×32	25.0	67	1.5
33	22.5	×69	0.8
×34	20.0	×72	0.4
35	19.4	×75	0.2
36	18.8	×81 (no digestion)	0.0

N. B. Y = % of enzymic solution.
× = experimental value.

This table can be used for amylases from molds and grains, as the experiment with malt amylase showed almost same result. If a viscosimeter of different flowing velocity is used, then this table can be used by calculating relative velocity.

This method is quite convenient and accurate, and is used by the authors for the comparison of many kinds of germinated and ungerminated grains and molds.

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ABSTRACTS FROM THE ORIGINAL PAPERS.

The Effect of Acid Hydrolysis upon ~~S~~Cystine, and the Determination of Cystine in Protein.

(*The third paper on Sulphur Containing Amino-acids.*)

By YUZURU OKUDA and JUNJIRO MOTOMURA.
From the Biochemical Laboratory, Department of
Agriculture, Kyushu Imperial University, Fukuoka.

1. The effect of acid hydrolysis upon cystine:— It seems that the first important question to be solved for the determination of cystine in proteins is whether cystine is decomposed or not during acid hydrolysis. Van Slyke (J. Biol. Chem., **10**, 38, 1911) found that when cystine was boiled with 20% HCl for 24 hours, only 50% was precipitated by phosphotungstic acid, and he stated that it appears possible that the cystine is partially destroyed during the hydrolysis. Plimmer (Biochem. J., **7**, 311, 1913) also found that cystine is decomposed by boiling with conc. HCl for 5–8 hours. From these descriptions we see that cystine is pretty unstable when boiled with mineral acid. If it is true, it is difficult to determine cystine in proteins after acid hydrolysis. But on the contrary Gortner and Holm (J. Am. Chem. Soc., **42**, 821, 1920) found that cystine was not readily deaminized. Namely they boiled some amino acid mixture containing cystine with 20% HCl for 24 hours, and showed that if all of the ammonia nitrogen was calculated as being derived from the cystine, only less than 3% of cystine has been changed. Lately Hoffman and Gortner (J. Am. Chem. Soc., **44**, 341, 1922) also determined cystine, after prolonged boiling with HCl, by Okuda's bromine method (J. Coll. Agr. Imp. Univ. Tokyo, **7**, 69, 1919), and came to the conclusion that there is no appreciable decomposition

of cystine during ordinary acid hydrolysis of proteins, but the amount of cystine precipitable as phoshotungstate decreases, as a part of the cystine is transformed to its isomer, whose phosphotungstate is readily soluble.

In this present paper the authors have ascertained the results obtained by Gortner and his co-workers. In the first experiments, pure cystine was boiled with 20 % HCl, or 25% H_2SO_4 respectively, for 30 hours and cystine was determined in the solution by the bromine method. In the second group of experiments some human hair was hydrolysed by boiling with fifteen times its weight of HCl in different concentrations as 20, 25 and 38%. After a certain time from 15 to 30 hours, of boiling under a reflex condenser, 10 c.c. of the solution was withdrawn and cystine was determined by Okuda's iodine method (J. Chem. Soc. Japan, **45**, 18, 1924), and Folin and Loony's colorimetric method (J. Biol. Chem., **51** 421, 1922). In every experiment performed, no appreciable decomposition of cystine was observed during the time of acid hydrolysis.

2. The determination of cystine in proteins.– The bromine method is a very rapid and accurate method for the determination of cystine, but it can not be used directly in the presence of tyrosine and tryptophan. Therefore we used the iodine method which is available for the determination of cystine in the presence of all the cleavage products of proteins. Folin–Loony's colorimetric method was also used for the sake of comparison. The two methods gave similar results in the proteins such as hair, wool, edestine, peptone and several kinds of muscle proteins, but in some proteins such as nuclein, casein, gelatine etc., which contain every small quantity of cystine, the colorimetric method gave much larger results than those of the iodine method. Especially was this the case with pure nucleic acid, guanine sulphate and glucose which, though they gave no csytine after acid hydrolysis by the iodine method, gave an appreciable amount of cystine by the colorimetric method. The authors came to the conclusion, therefore, that colorimetric method can not be used in some proteins, as there are some substances, other than cystine in the hydrolysed solution, which produce the color reaction like cystine.

3. The iodine method:– Although this has already been published by one of the authors (Okuda) in Japanese, for the sake of the reader's convenience we shall describe it here briefly. The basis of the method depends upon the fact that among the amino acid derived by the hydrolysis of proteins cysteine alone reacts very actively to the iodate–iodide–hydrochloric acid mixture in an acid solution. The principle

of the method is to titrate cysteine, freshly produced from cystine by reduction, with a standard iodate solution in the presence of some iodide and hydrochloric acid.

For the determination the following solutions are required :- (1), About 5% KI aqueous solution. (2), Exactly 4% HCl. (3), Exactly 2% HCl. (4), M/300 KIO_3, which is prepared by dissolving 2.14 g. of pure KIO_3 in 3 liters of exactly 2% HCl. This iodate solution should be standardized for cystine very carefully. Standardizing :- Dissolve 1.01 g. of cystine in 50 c.c. of about 5% HCl, add a few decigrams of zinc dust, leave it for 30 minutes at a room temperature (about 20° C), shake it now and then. Filter, wash and make it up to 100 c.c. with water. Take 1 c.c: of the freshly prepared filtrate immediately in a small dry Erlenmeyer flask or in a large test tube, mix with 19 c.c. of exactly 2% HCl, 5 c.c. of 5% KI and 5 c.c. of exactly 4% HCl, and then titrate with the iodate solution until a yellow color is produced. Then the required volume (4.65 c.c. at 17.5° C.) of the iodate solution corresponds to 0.0101 g. of cystine. The coefficient (c.c. of KIO_3) differs in different temperature, therefore it is conveninent to make a temperature curve in the beginning of experiments.

Procedure :- Take 1—10 g. of protein, boil it with about three times its weight of conc. HCl (sp. gr. 1.19) under a reverted condenser for about 20 hours. Decolorize it with the best charcoal by heating for 30 minutes, and then filter and wash with some boiling water. Add a little zinc dust to the filtrate for the reduction of cystine to cysteine, and leave it for 30 minutes at a room temperature. And then filter, wash and make it up to 100c.c. Take 1c.c. of the solution for the determination of the concentration of HCl in it, by means of the titration with a standard alkali solution. To the residual solution, add a calculated quantity of 20% KOH (or 20% HCl) to make it into a solution containing exactly 2% of free HCl. After ascertaining by titration that the solution contains exactly 2% HCl, take a definite volume (less then 20 c.c.) of the solution in a small dry Erlenmeyer flask, make it up to 20 c.c. with the exactly 2% HCl. Add 5 c.c. of 5% KI and 5 c.c. of 4% HCl, and then titrate with the standard KIO, solution, until the yellow color produced remains for one minute. If the temperature of the mixture was 17.5° C., and the iodate solution has been previously prepared so that its 4.65 c.c. corresponds to 0.0101 g. of cystine, the cystine content in the final 20 c.c. is as follows :-

0.0101 × required volume of KIO_3/4.65.

In this method the sample for a single determination should contain 2% HCl and 0.005—0.05 g. of cysteine in 20 c.c. of the solution. Especial attention is called to the fact that both the standardization of the iodate and the determination of the cystine must be made with an equal concentration of HCl (e.g, 2%), and with freshly reduced solutions. As cysteine is autooxidizable even in an acid solution, the reduced solution must be titrated within two hours.

If the original hydrolysed solution contains some cysteine, the results obtained as above express the sum of cystine and cysteine. But all the hydrolysed solutions investigated have contained no cysteine. For the purpose of testing cysteine in the hydrolysed solution, add a few c.c. of KI and a drop of the iodate. In the presence of cysteine the solution remains colorless, but in its absence it gives a yellow color. This reaction is more sensitive than the well known nitroprusside-reaction.

If cysteine is present in the hydroysed solution, the separate determination of cystine and cysteine is easily accomplished by the above method, titrating a sample solution before and after the reduction, and calculating the difference of the two results. In this case 4.65 c.c. of the iodate solution corresponds to 0.01 g. of cysteine, which will be derived from 0.0101 g. of cystine by the reduction with zinc and HCl.

The Cystine Content of Muscle Proteins of Marine Animals.

By ZEMPEI OGURA and KŌ FUJIKAWA.

The cystine content of various muscle proteins of marine animals was determined by means of the iodine method described by Okuda, with the following results:—

Sample	Cystine in 100 g of moisture free proteins
Fish,	
Scomber japonicus	0.82
Spheroides sp	0.87
Raja sp	0.98
Squolus japonicus	0.95
Mollusc,	
Stichopus japonicus	0.75
Haliotis sp	0.62
Arca inflata	0.59

Polypus octopodia	0.92
Cardium muticum	0.72
Crustacea,	
Neptunus pelagicus	1.12
Penaeus canaliculatus	1.19
Chionoecetes phalangium	1.19

From the results of the analysis we see that the muscle proteins of crastacea contain much more cystine than those of the other marine animals. This fact seems to have some relation on the cause of the black-changing of the flesh of crab and lobster, which sometimes takes place in the cases of canning and cold storage.

Studies on the Enzyme Action.

I. On Phytase.

By MATSUNOSUKE KITAGAWA.
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Kyushu Imperial University, Fukuoka, Japan.

The phytase solution and phytin were prepared by the following method:— For the preparation of phytase some rice bran was extracted with seven times its weight of 25% alcohol. To the extract one tenth its volume of saturated barium hydroxide and basic lead acetate solution were added, and the clear filtrate thus obtained was dialysed in a collodion membrane for two days. Such a phytase solution was more active than that prepared by the adsorptionmethod; it was pretty pure, containing no phytin and phosphate, and only minute traces of protein. The activity of phytase in water solution did not decrease for more than a week.

For the preparation of phytin, the crude phytin obtained from rice bran by the usual process was dissolved in 2% HCl, precipitated by barium hydroxide and washed several times with some hot water. The barium salt of phytin was again dissolved in 2% HCl, Ba removed by means of 6% H_2SO_4 quantitatively. To the clear filtrate some absolute alcohol was added and phytin was precipitated as a viscous mass, which was dried in a desiccator.

The phytin so prepared was very readily soluble in water, slightly acidic, and contains 49.5% P_2O_5.

For the estimation of phosphate, Brigg's modification of Bell and Doisys' colorimetric method (J. Biol. Chem., **53**, 13, 1920) was used.

At first the optimal pH for the enzyme action was determined in several buffer solution.

Phytase was less active in the citrate mixture than in the acetate or lactate mixture, but the optimal pH was the same in each case being 4.6—4.7. (Such a concentration of citrate as examined had no influence on the estimation of phosphate).

To a large extent, the concetration of phytase is exactly proportional to the amount of the phosphate produced, but in a very low concentration, the activity is not parallel with the concentration of the enzyme, but very much diminished.

There is a certain range of the optimal concentration of the substrate for the phytase reaction. Such an optimal range depends upon the concentration of enzyme, but not on the reaction stage and nature of the buffer mixtures which influence the action of the enzyme.

When the concentration of substrate is lower than the optimal, the time curve in the case of phytase reaction is logarithmic in nature as usually obtained, but in the optimal concentration the reaction velocity is independent of the concentration of the substrate and the curve becomes a straight line.

Studies on the Acids formed by Rhizopus species.

By TEIZŌ TAKAHASHI and KINICHIRŌ SAKAGUCHI.

On the nature of the acid formed by the species of Rhizopus K. Saitō has mentioned already in 1904 in the description of his Rh. chinensis, (Cent. f. Bact. II. 1904, **13**, p. 54). Seven years later, Fe. Ehrlich found fumaric acid in the culture of Rhizopus nigricans in the medium containing glycocoll as its nitrogen source and this is the first instance ever since made on the formation of this acid from Rhizopus. He obtained oxyphenyl-lactic acid in the culture of the medium containing tyrosine instead of glycocoll, (Ber. 1911 **44**, Bd. III. S. 3737). In his second report, he mentions beside fumaric acid a trace of volatile acid, succinic acid, *l*-malic acid and *d*-lactic acid and moreover he adds that the some acids were affirmed to be produced by Rhizopus tritici Saitō. (Ber. 1919, Bd. **52**, S. 63).

On the other side, Goupil in 1911, isolated Succinic acid from the culture of Rhizopus Rouxii (Amylomyces Rouxii) (C. r. 1911, **153**, p. 1172—1174).

M. Hanzawa (Mycol. cent. 1912, Bd I, S. 76—92 et. 1914, Bd V. Heft. 5, S. 230, et. 1915 V. Heft. 6, S. 272—279). M. Yamazaki (Report of "Tōadōbunshoin" in Japanese. 1911 et 1912), R. Nakazawa (Rep. of Gover. Resea. Inst. Formosa, Japan) and recently Y. Takeda (Rep. of Gover. Resea. Inst, Formosa, Japan. No. 5 of the Depert. of Indust. 1924). have studied on the formation of the acid by their Rhizopus but left aside the research of its nature.

The writers made researches on the formation and isolation of the acids formed from 17 species of Rhizopus and confirmed that we could devide these species into three types:- The first type forming fumaric acid mainly and none of lactic acid. The second type chiefly forming lactic acid and none of fumaric acid. Third one forms both fumaric and lactic acids.

The first type beside fumaric acid accompanies with a traces of citric, malic and tartaric acid (?). All species of the three types produce a trace of volatile acid and special species (Rhizopus chankuoensis) seems to form a trace of succinic acid beside fumaric acid.

To the first type twelve species of them such as Rhizopus Oryzae, R. tonkinensis vullemin, R. formosensis Nakazawa, R. formosensis var. chlamydosporus Yamazaki, R. candidus Yamazaki, R. chankunnensis Yamazaki, R. Hangchow Yamazaki, Rhizopus G. 34 Yamazaki, R. chiuniang Yamazaki, R. Delemar Wehmer et Hanzawa, R. niveus Yamazaki, R. albus Yamazaki are to be mentioned.

The second type involves:- Rhizopus salebrosus Yamazaki, Rhizopus G. 36, Yamazaki.

The third type implies three species:- R. chinensis Saito, R. liquefaciens Yamazaki, R. pseudochinensis Yamazaki.

The necessary and satisfactory conditions for the maximum formation of the fumaric acid is the ratio of nitrogen to carbon source in the nutrient. It is about 0.01 : 72 so in practice the most adequcte quantity of the former in the nutrient is adout one tenth of the common medium.

As the nitrogen source urea, zlycocoll, as Ehrlich has mentioned before, may be used as good one, but also gelatine, peptone, can play the some role as these and even inorganic nitrogen compound such as NH_4NO_3, $(NH_4)_2SO_4$ may be used as good as organic nitrogen under the rule mentioned above.

Glucose, cane sugar, and starch are very good carbon sources for the growth as for as well as the formation of fumaric acid.

The culture medium used was prepared as follows; water 1000 c.c., carbohydrate (glucose, cane sugar or starch) 100g., K_2HPO_4 0.15g, KH_2PO_4 0.15g, $MgSO_4$ 0.1g, $CaCl_2$ 0.1g, Fe_2Cl_6, NaCl trace, nitrogenous matter (peptone, urea, or gelatine) 1g, 1.2g, or 1g. and $CaCO_3$ 50g. (in some case this salt was omitted).

Isolation of Acids.

Fumaric acid: In the case when starch is used in the absence of $CaCO_3$ in the medium, for the isolation of the acid the concentration of the culture just by evaporating it is enough to get it in a free state, after the separation of mould growth from the culture on behalf of very tiny solubility of the acid. If $CaCO_3$ was added to the medium the acid becomes naturally into calcium salt, so calcium is removed from it by dilute sulphuric acid as sulphate. The filtrate from Ca-sulphate is acidified with sulphuric acid and the free acid is extracted by ether in Sudō-Kumakawa extraction apparatus. Raw acid, after recrystalisation from hot water or hot alcohol is purified. In the mother liquor citric, malic and succinic acid may be detected.

Lactic acid: This acid may be isolated in the same way as in the case of fumaric acid.

If both fumaric and lactic acid occur in a mixed state they are separated as lead salts or barium salts; where just lead lactate is soluble in water or barium-lactate is saluble in 70—80 vol% of alcohol, when hot.

Identification.

Fumaric acid thus obtained sublimes at 200° C, melts at 285° C in a sealed tube. (After Fe. Ehrlich 280°).

The number of titration, substance taken 0.1g, 17.3 c.c. found, 17.24 c.c. calcul.

The hydrogen centents by elemental analysis;

Substance taken		0.2058 g
H_2O		0.0659 g
H	found	3.55 %
H	calculated	3.45 %

Silver salt. The silver was determined as chloride with the data of:—

	Substance taken.	Agcl.	Ag.	
$Ag_2C_4H_2O_4$ (dried at 110° C)	0.152 g	0 1314	found.	65 38 %
			calcul.	65.44 〃
$Ag_2C_4H_2O_4$ (dried at room t°)	0.0622 g	0 0515	found.	62 32 〃
$+H_2O$.			calcul.	62.04 〃

Dimethylester, $(CH_3)_2. C_4H_2O_4$ prepared from silver salt and methyliodide melts at 102° C. (Anschütz, 102° C. B. 12, 2282, 1879).

Dibrom–succinic acid, prepared by the addition of bromine to the substance melts at 255—256° C.

l–Lactic acid.

The acid from Rhizopus G. 36.

Zn–salt, water of crystallisation 12.18% (12.89% after Hoppe Seyler's Hand buch . S. 77. for active salt).

Zn–salt $[\alpha]^{15°} = +6\ 87$(4% salution)

The same from Rhizopus salebrosus.

Zn–salt. water of crystallisation 13.03%.

〃 $[\alpha]_D^{15°} = +6.25$(3 6% solution).

(After Hoppe–Seyler and Araki's result with *d*–lactated of 4.18% solution $[\alpha]_D = -7.55$ and of 9.08% solution $[\alpha]_D = -6.56$)

From the figures given above this acid must be a laevo–rotatory ones.

For citric, malic and tartric acid just a qualitative test was made, since the quantity was not enough. The cryitals obtained from the mother liquor of fumaric acid was taken, gave a precipitation of Hg–acetondicarbonate after Denige, which is a characteristic reaction of citric acid. (Abderhalden. Handbuch Arbeitm. Bd. II. S. 33). The same mass after neutralisation reduced palladium chlorid after the method of A. Hilger and H. Ley. (zeit. f. d. Unt. u. Nahr. u. Genus. Bd, 2. S, 795, 1899) This is characteritic to malate.

The same mass gave a violet red coloration when treated by resolcimol solution in sulphuric acid. This reaction is characteristic far tartric acid. (Roy. C. r. 1907, 145, 1285).

For the identification of these three acids a further researches is going on and its will be reported in future.

Sinomenine and Dehydrosinomenine.

Part III.

By KAKUJI GOTŌ.

The method of kali fusion of sinomenine was improved, and the yield of the substance of m.p. 176° (formerly it was given as 169°, but the new m.p. was attained through the recrystallisation from acetone.) amounted to 30% of the theoretical.

The substance of m.p. 176° has the molecular formula $C_{16}H_{16}O_4$, contains two methoxyl groups and shows the reaction of a dipuenol, reminding particularly of the reaction of orthodiphenols such as apomorphine and pyrocatechine. The original carbonyl group contained in sinomenine seems to have been modified in this substance into a phenol group. I propose, therefore, to call this new substance sinomenol.

Sinomenol forms dibenzoyl (m.p. 207°) and diacetyl (m.p. 147°) derivatives. Methylation of sinomenol in an alkaline medium with dimethylsulphate leads to two substances (m.p. 115° and 240° respectively). Both the methylated sinomenols give no colour reaction of the original substance, have the same molecular formula $C_{18}H_{20}O_4$ and contain four methoxyl groups, estimated by the method of Zeisel. Dibenzoylated sinomenol melts at the same degree as the benzoylated substance obtained directly from sinomenine by the action of benzoylanhydride (see, this Journal 1924, vol. 1, p. 10 ; there the moleculer formula was errornously given as $C_{25}H_{2}O_5=5C_5H_4O$, but it is now ascertained from various analysis that it must have the formula $C_{30}H_{24}O_6=6C_5H_4O$. The admixture of these two substances did not change the melting point. There is, therefore, no dout about the fact that the latter substance is nothing but dibenzoylsinomenol. This fact was, moreover,' confirmed by the debenzoylation of the latter substance, which gave sinomenol itself.

Diacetylsinomenol (m.p. 149°, the formula, $C_{20}H_{2}O_4$) is easily hydrolysed by alkali and then shows every colour reaction of sinomenol. It was found that the same diacetylsinomenol could be obtained directly from sinomenine by heating it with five parts of acetanhydride in a sealed tube for six hours at 175°.

Concordance of the results of elementary analysis of sinomenol, diacetyl, dibenzoyl and dimethyl sinomenols as well as dibenzoylsinomenolchinone leaves no doubt about the fact that sinomenol contains no vinyl side chain. The negative result obtained in an attempt to oxidise sinomenol in an acetone solution with permanganate also confirms this idea (ibid. p. 11).

Destillation of sinomenol with zinc dust gave crystals, which melted at 95°. It is almost certain that it is phenanthrene, since its picrate melts at 143° scharply.

From these facts sinomenol seems very likely to be a dioxydimethoxyphenanthrenedihydride.

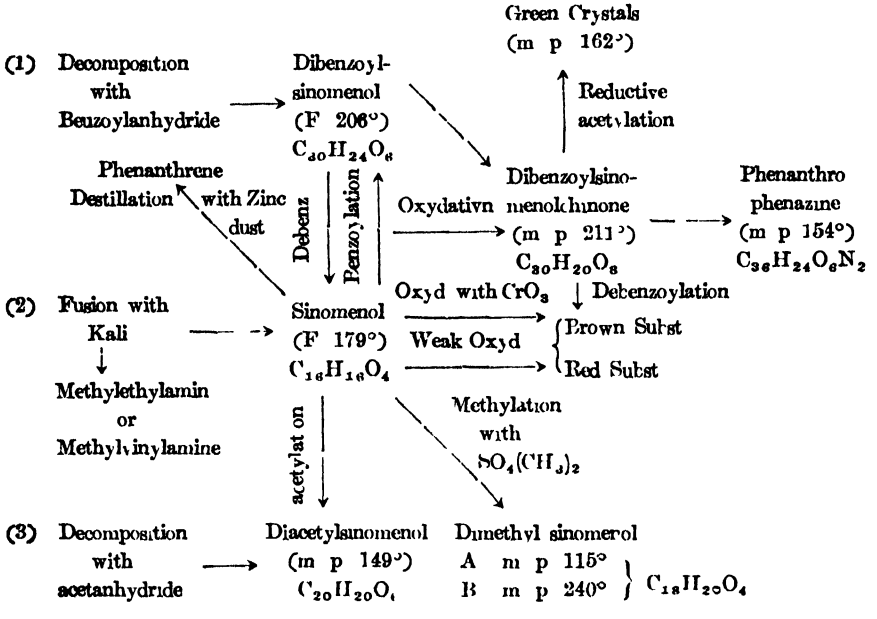
If sinonenol contains no vinyl side chain, then the chain of two carbon atoms must have been detatched from the phenanthrene nucleus together with the methylated nitrogen atom in the operation of the fusion with kali. It would then give rise to methylethanolamine or allied substance. The amine actually isolated was certainly not methylamine, and from the m.p. of the auri-and platinichloride, as well as the content of gold and platinum of these double salts, it is suspected to be methylethylamine or methylvinylamine.

Very striking is the ease with which sinomenol undergoes oxidation. Poured with 10% caustic soda and well stirred, it dissolves in it, taking at the same time a beautiful violet colour. The same colour is produced when sinomenol is oxidised with a very small quantity of permaganate, ammoniacal silver solution or alkaline ferricyanide. On the contrary, acidic oxidizing agents such as nitric acid, nitric acid and silver nitrate and gold chloride give a deep red colour to the acetone solution of sinomenol. These changes seem to be one and the same. For, from the violet aqueous solution a red precipitate is thrown down on addition of an acid and is extracted then by organic solvents such as ether. From the alkaline violet aqueous solution neither ether nor chloroform can extract the substance, so it seems that in the violet solution there is the formation of salt of the oxidised substance.

As dibenzoyl and dimethyl sinomenols give no such reaction, the two hydroxyl groups of the sinomenol must be in a direct relation with the formation of this coloured substance. The deep colour reminds us of the existence of a chinone. But the mild reagents such as air oxygen or ammoniacal silver solution would not be able to attack the carbon atoms in the positions 9 and 10 in phenanthrene nucleus. It seems, therefore, very likly that the two hydroxyl groups in sinomenol lie in an orthoposition each other. Moreover, there is probability that a new hydroxyl is introduced (as is the case with 1—8 dioxynaphtaline), otherwise it would not be able to form a violet salt. This was indirectly proved by the fact that the reductive acetylation of this red substance leads to an acetyl compound entirely different (m.p. over 180°, as yet amorphous) from the diacetylsinomenol.

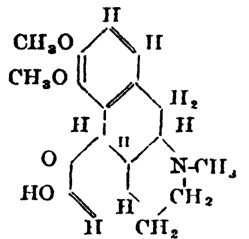
The oxidation of sinomenol with chromic acid in glacial acetic acid gives a small quantity of the above red substance and much of a brown substance, which is very insoluble in this solvent. This substance seems to be very likely the same substance with that obtained by the alkaline hydrolysis of dibenzoylsinomenolchinone. Neither the red substance nor its derivatives have not yet been obtained in crystals.

The relation of the above given substance each other can be seen clearly from the following table.

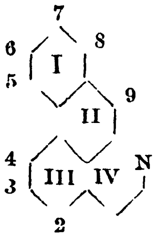


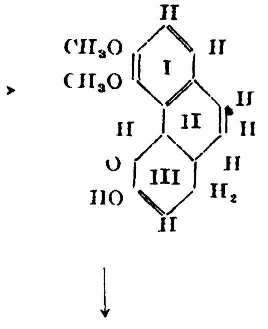
On the constitution of Sinomenine.

It is now clearly established that sinomenine ($C_{19}H_{23}O_4N$; with two methoxyls, one carbonyl and N-methyl) is a tetrahydroisoquinoline alkaloid belonging to a phenanthrene group. Its constitutional formula may be given provisionally as follows.

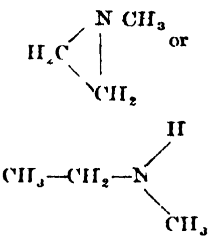


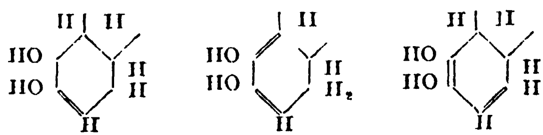
I Sinomenine



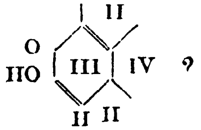


Methylvinylamine or Methylethylamine

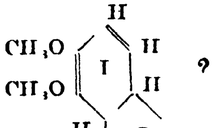




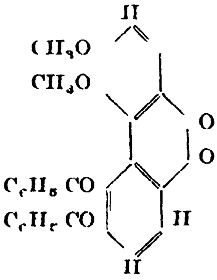
II Sinomenol



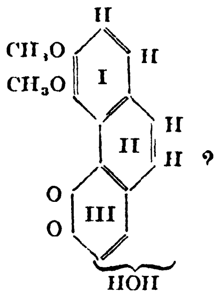
Dehydrosinomenine



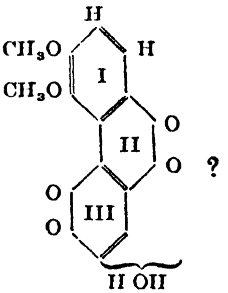
Hydrosinomenine



Dibenzoylsinomenol quinone



Red Substance



Brown Substance

The position of the two methoxyls and the benzenoid character of the nucleus, to which these two methoxyls are attached, have been selected hypothetically according to the constitution of the most of the opium alkaloids.

The position of the carbonyl (4) and of the hydroxyl (3) was selected according to the behaviour of the sinomenol and the red substance derived from it. For, if sinomenine has the foumula I, then sinomenol must have the formula II.

This explains well the formation of dibenzoyl, diacetyl and dimethyl derivatives of sinomenol. That the methylaminoethyl group ($-CH_3N-CH_2-CH_2-$) is attached to this skeleton in positions 10 and 1 (and particularly N in 10) is deduced from the following facts. Benzoylsinomenine contains only one benzoyl group (J. of chem. Soc. of Japan, 1924, June number), and sinomenine when methylated, takes only one methyl group and this monomethylsinomenine still forms an oxime (this point is not yet proved by the auther. But compare. Kondo and Ochiai, J. of pharm. Soc. of Japan, 1923, No. 503. p. 20) These facts show that the paraposition of the carbonyl group in sinomenine is occupied, so as it gives hindrance to the desmotropic change of the carbonyl group, observed in sinomenol. (The alternative that N is attached at the position 1 and C in 10 is very unlikely from the known facts about the phenenthrene alkaloids. But the decisive conclusion can be given only when sinomenol carboxylic acid is obtained)

The formation of the red substance from the sinomenol makes us believe that the original carbonyl and the hydroxyl must lie in an orthoposition each other. And the fact that sinomenine gives an intense diazoreaction requires that the ortho-position of the hydroxyl must be free. Hence the hydroxyl in position 4. The unsaturation between 2 and 3 is assumed from the phenolic character of the same hydroxyl.

Although the above formula holds good in main respects, it has naturally its difficult sides also. For, the formation of dehydrosinomenine ($C_{19}H_{21}O_4N$) is well explained, if we assume by the dehydrogenation the hydrogen atoms marked with asterisk are taken away. But how it can account for the formation of hydrosinomenine ($C_{10}H_{25}NO_4$) ? This substance gives also an intense diazoreaction as well as ferric chloride reaction, so that the unsaturation between 2 and 3 must remain untouched. Will the benzenoid nucleus be reduced partially by moleoular hydrogen and colloidal palladium ? This is somewhat singular, but no other explanation can be put forward easily.

The relative position of bromine atom in two different isomeric monobromsinomenines (ibid. p. 8.) is not yet more than imagination. The isomer of lower m. p. (153°), which gives ferric chloride and diazoreaction, may contain the bromine atom in the nucleus I, and the higher m. p. (214°) may contain it in the position 2 of the nucleus III. Yet it does not explain why the ferric chloride reaction disappears also in this isomer.

The decision whether the above formula is to be regarded correct or not may be given in a degree by the oxydative decomposition of the sinomenol or its derivatives. This is my next task and will be, I hope, reported shortly after.

EXPERIMENTAL.

Sinomenol, by kali fusion of sinomenine. 10 grs. of sinomenine, 100 grs of potassium hydroxide and 50 grs of water were heated in a distilling flask on a glycerine bath at 180-200°. The liquid distilling over was collected in a test-tube, and the gas was caught in two per cent hydrochloric acid. After two hours heating, the content of the flask was made acidic with 15% sulphuric acid and extracted twenty times with ether. When the ether was dried and distilled off, sinomenol crystallised out in the red substance, which could be washed out with methyl alcohol. It can be recrystallised from glacial acetic acid, ethyl alcohol or acetone, but the oxidation sets in sometimes during the operation. M.p. 176°. Yield 2.4 grs., i.e. 30% of the theoreti cal. It has the formula $C_{16}H_{16}O_4$ (C=70.39% ; H=6. 3% ; $C_{16}H_{16}O_4$ requires C=70. 58% ; H=5.89%.) and contains two methoxyl groups (20.73% ; calculated, 22.79%,) For the colour reactions of sinomenol, see the theoretical part.

Sinomenol, by debenzoylation of the dibenbzoylsinomenol obtained directly from sinomenine by benzoylanhydride. 0.6 gr. of the dibenzoylsinomenol was boiled with 9 grs of 66% caustic kali about fifteen minutes. The solution was made acidic amd extracted five times with ether. The residue, when the ether was evaporated, was washed with a small quantity of methyl alcohol to free it from benzoic acid. Yield 0.1 gr. M. p. 176°. (unaltered by the admixture of sinimenol obtained by the kali fusion).

The methyl alcoholic filtrate is coloured intensely red. This shows that the red substance can also be formed from sinomenol or rather, that the red substance formed in the kali fusion of sinomenine is perhaps the same with that obtained by the oxidation of sinomenol, so that the red substance is formed secondarily from sinomenol in the kali fusion of sinomenine.

Diacetylsinomenol, by the acetylation of sinmenol. 1.5 grs of sinomenol, 3 grs. of anhydrous sodium acetate and 20 grs. of acetanhydride were boiled for six hours with air condensor. Poured into water, diacetylsinomenol separated out in crystalline powder. Recrystallised from fifteen parts of methyl alcobol, it crystallises in beautiful prisms. M. p. 149°. It has the molecular formula $C_{20}H_{20}O_6$ (C=67.55% ; H=5. 93% ; $C_{20}H_{20}O_6$ requires C=67.41% ; H=5.62%) and contains two acetyl groups(22. 76%, as the result of hydrolysis by cold sodium ethylate; calc. as two acetyl groups 24.15%). It is hydrolysed by 10% caustic soda and then shows the colour reaction of sinomenol.

Diacetylsinomenol, by the action of acetanhydride on sinomenine. 2 grs of sinomenine mixed with 10 c.c. acetanhydride were heated in a sealed tube at 175° during six hours. The brown solution was poured in water and well stirred. Crude yellow powder thus ontained amounted to 2 grs. Twice recrystallised from glacial acetic acid the yield was 0.4 grs, thus ca. 20%. of the theoretical. M.p. 149°. Admixture with the diacetylsinomenol obtained by the acetylation of sinomenol did not alter the m.p. The scanty yield is perhaps due to the difficulty of the isolation.

Dibebenzoylsinomenol by debenzoylation of sinomenol. Sinomenol seems not much to be benzoylated by heating it with benzoylanhydride in boiling water-bath. Better it is benzoylated in ethylbenzoate with benzoylchloride and caustc alkali after the manner of Schotten-Baumann. Recrystallised from glacial acetic acid, it melts at 207°. Yield ca. 70% of the theoretical. Mixed with the dibenzoylsinomenol obtained directly from sinomenine by benzoylanhydride, its m. p does not change. Other proprties as well as the analytical results are identical in the both samples.

Analysis. C=75.48% ; H 5 27% ; $C_{30}H_{24}O_6$ requires C=75.00% ; H=5.00%.

The hydrolysis of the dibenzoylsinomenol is not easy. Weak alkali does not attack it, while the strong destroys the molecule of sinomenol and give rise to volatile acids. But from the results of the above analysis and the behaviour of the other derivatives of sinomenol, it is certain that it contains two benzoyl groups.

Dibenzoylsinomenolchinone from the sinomenol obtained by kali fusion. Sinomenol was benzoylated as above and oxidised to chinone The intense reddish yellow colour revealed at once that it is the same substance with that produced from dibenzoylsinomenol obtained directly from sinomenine by the action of benzoylanhodride. M. p. 211°. The mixture of the both samples melted at the same degree. (Note. The formula of this chinone now must be $C_{30}H_{20}O_8$)

Reduction of dibenzoylsinomenolchinone. When dibenzoylsinomenolchinone is boiled with glacial acetic acid and zinc dust, its reddish yellow colour disappears quickly, showing that reduction is going on. But on filtering, it takes a deep green colour and then recovers instantly its original reddish yellow colour. The crystalls, which were formed on cooling, were reddish yellow and meltod at 211°.

The deep green colour is perhaps given by the formation of chinhydrone like substance in the way of autoxidation of the reduced chinone. The latter was fixed by the reductive acetylation.

Reductive acetylation of dibenzoylsinomenolchinone. 0.2 gr. of this substance was reductively acetylated by boiling it with 5 grs of acetanhydride and 0.5 gr of zinc dust for six hours. The precipitate, formed on addition of water, was recrystallised from methyl alcohol. It forms fine prisms, collected in rosettes. M. p. 164°. Analysis is not yet performed on account of the scarcity of the substance.

Methylation of sinomenol. 2 grs of sinomenol were methylated with 30 grs of 10% caustic soda and 10 grs of dimethylsulphate, added in four portions. After standing overnight, it was diluted with water, filtered and the residue was washed with water, until the filtrate showed no longer alkaline reaction. The residue was extracted with boiling methyl alcohol. On evaporating methyl alcohol, slightly coloured crystals were obtained. M. p. 115° (not sharp). It has the molecular formula $C_{18}H_{20}O_4$ (C=72.59% ; H=6.51% ; $C_{18}H_{20}O_4$ requires C 72.00% ; H=6.71%). and contains four methoxysl(40.36% ; calc. 41.33%).

The residue, which was insoluble in boiling methyl alcohol, was extracted with boiling glacial acetic acid, from which beautiful needles crystallised out. M. p. 240°. It has the same molecular formula as the above. (C=72.01% ; H=6.39%) and contains four methoxyls also (40.71% ; calc. 41.33%).

The both substances dissolve in concentrated sulphuric acid with brown colour and it turns green when slightly warmed. But on addition of water a striking difference is observed. The substance of m. p. 115° decolourises at once, while the substance of m. p. 240° takes a beautiful purple colour, giving a reddish amorphous precipetates after a time.

The reason, why these two methylated substances are produced, is not yet accounted for.

Distillation of sinomenol with zinc dust. 1 gr of sinomenol was distilled with much zinc dust in a tube, in an atmosphere of hydrogen. 0.1 gr of crude crystals was obtained, which, recrystalliied from alcohol melted at 95°. Its picrate melted sharply at 143°, so it is almost certain that we have phenanthrene here.

Nitrogenous decomposition products. In the kali fusion of sinomenine, amount of the escaping gas, which was absorbed in dilute hydrochloric acid was very small. On evaporating the hydrochloric acid solution, only a thin crust was observed on the bottom of the watch-glass. This part was, therefore, thrown away.

The liquid condensed in the test tube was filtered and extracted five times with ether. The ethereal extract was shaken with dilute hydrochloric acid and the latter was evaporated.

The aqueous part was also neutralised with hydrochloric acid and eveporated. From these two portions the platinichlorides were prepared separately and their m. p. was compared. As both these platinum double salts melted and decomposed sharply at 224°, it was clear that we had the same amine in the ethereal extract as well as in the aqueous solution. The gold double salt melted at 179-180° without decomposition. The content of Pt and Au was respectively 37.28% and 49.35%.

From the content of Pt and Au in these double salts. it may be inferred that we have here methylethylamine or methylvinylamine. The m. p. of the gold double salt coincides with that of methylethylamine aurichloride, but the m. p. of the platinichloride shows the difference of twenty degrees from that of the methylethylamine platinichloride. As regards the suspected methylvinylamine it seems that this amine has not yet been described in the literature.

The hydrochloride of this amine is very hygroscopic and deliquates in a few minutes in the air and its m. p. can not be derterminad easily.

The theotetical yield of this amine from 55 grs of sinomenine, based on the yield of sinomenol is 2.97 grams. Calculated from the amount of hydrochloric acid required to neutralise the alkaline destillate, the actual yield seems to be not more than 2.5 grs. So it may be concluded that almost all the amine produced when sinomenine decomposed into sinomenol was collected in this destillate. When the sulphuric acid solution, from which sinominol was isolated, was evaporated down to a small volume, made alkaline and distilled, it gave only a small quantity of basic substance.

The red substance. The red substance is formed as stated above when sinomenol is exposed to the action of milder oxydising agents. It is also formed at the time of kali fusion of sinomenine. It is not yet obtained in crystalline form.

The red substance is very sensitive to the nitric acid. Warmed with a small quantity of the latter, it turns yellow and a faint aromatic odour is set out.

The red substance dissolves green in concentrated sulphuric acid, but on addition of water a red amorphous substance is again precipitated.

The red substance is best prepared by the action of ammoniacal silver solution on sinomenol. 1.5 grs of sinomenol are dissolved in 100 c.c. of alcohol, added with alcoholic solution of 3 grs of silver nitrate and then made alkaline with concentrated ammonia. After several hours the violet solution is made acidic and filtered. The silver is washed well with alcohol. The combined alcohol is poured into ten volumes of water. The red amorphous precipitate thus obtained is collected and washed. Yield ca. 0.6 gr, i.e. 30%.

Acetylation of red substance. The red substance was acetylated as with sinomenol. Yellow crystalline powder was obtained, which melted at about 80°. It is easily hydrolysed by alkali and then on acidifying, it recovers its original red colour.

Reduction of the red substance. The red substance is decolourised by glacial acetic acid and zinc dust. But on standing it recovers its colour after some time so that the reduced substance can not be isolated in a usual way.

Reductive acetylation of the red substance was carried out in a way exactly similar to that of dibenzoylsinomenolchinone. Yellow crystalline powder was obtained which was quite differet from diacetylsinomenol and melted at about 180°. This shows perhaps that a new hydroxyl was introduced in the red substance while the original hydroxyls were oxidised to a chinone group.

The brown substance from sinomenol by the oxidation with chromic acid. 0.4 gr impure sinomenol was oxidised in boiling glacial acetic acid with 0.2 gr chromic acid. On cooling 0.1 gr of the brown substance separated out. This substance is very difficultly soluble in methyl alcohol also. Judged from the condition of its fromation the brown substance is perhaps identical with the debenzoylated dibenzoylsinomenolchinone.

My best thanks are due to Prof. U. Sudzuki who has taken kindly interest in this work and given me constant encouragement

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TABLE II.

Food Consumption.

Average Daily Consumption of Ration per Animal.

Group No.	Date	Ration (gr)	Dry Matter (gr)	Na (mgr)	Na per 10 gr. body wt. (mgr)	K (mgr)	K per 10 gr. body wt. (mgr)
I	May 30–June 9, 1924	23.08	7.236	22.19	2.1	42.58	4.0
II	July 14—20	22.81	7.775	31.83	2.7	79.74	6.8
V	July 17—24	24.50	10.478	95.42	8.3	35.79	3.1
VI	July 21—27	21.38	8.200	62.02	5.4	93.96	8.2

The results of the previous experiment are shown for reference in Chart III.

(2) Metabolism.

To collect feces and urine the experimental rats were confined in a large glass dish measuring 21 cm. diameter by 24 cm. depth. The rats were supported about 3 cm. above by the double wire screen. By this means, the consumption of feces was practically entirely avoided. The experimental days during which excrements were collected were seven.

The results and calculations pertaining to the first, second, fifth and sixth groups of eight rats are given in Table III and IV.

TABLE III.

Metabolism of Sodium

Group No.	Date	Ratio of alkali meta's eaten Na : K = 1 :	Average daily total Sodium intake per rat (mgr.)	Average daily Sodium excreted per rat (mgr.)			Balance (mgr.)	Gain in weight (gr.)
				in feces	in urine	total		
I	May 30—Jun. 9 1924	1,918	22.19	9.89	20.12	30.01	−7.82	+14.0
II	Jul. 14—20 1924	2,505	31.83	13.10	25.23	38.33	−6.50	+ 2.5
V	Jul. 17—24 1924	0,375	95.42	14.65	79.10	93.75	+1.67	+ 6.8
VI	Jul. 21—27 1924	1,515	62.02	15.15	46.18	61.33	+0.69	+10.2

Table IV.

Metabolism of Potassium

Group No.	Date	Ratio of alkali metals eaten Na : K = 1 :	Average daily total Potassium intake per rat (mgr)	Average daily potassium excreted per rat (mgr.)			Balance (mgr.)	Gain in weight (gr.)
				in feces	in urine	total		
I	May 30—Jun 9 1924	1.918	42.58	4.67	35.35	40.02	+2.56	+14.0
II	Jul. 14—20 1924	2.505	79.74	6.95	70.13	77.08	+2.66	+ 2.5
V	Jul 17—24 1924	0.375	35.79	3.55	25.14	28.69	+7.10	+ 6.8
VI	Jul. 21—27 1924	1.515	93.96	5.00	85.43	90.43	+3.53	+10.2

(3) Metabolism of Nitrogen.

The contents of nitrogen in the rations used in this trial are shown in Table V. Almost all of the nitrogenous materials were furnished in the form of casein. Total nitrogen excreted in feces and urine were determined by Kjeldahl's method applying parts of samples which were collected for the determination of alkali metals. The results are shown in Table VI.

DISDUSSION

The alkali metals are absolutely necessary not only for growth of young animals but also for maintenance of health and the effect, attributed to their ratio, is more important, especially when the animals are very young.

Table V

Content of nitrogen in the ration

Ration	Total N in dry matter (%)
A	2.6741
B	2.6653
C	2.6657
D	2.6496

TABLE VI

Metabolism of nitrogen

Group No.	Date	Body weight av. (gr.)		average daily Consumption per rat		averge daily total nitrogen excreted per rat (mgr.)			average daily Balance of nitrogen (mgr.)	Apparent digestibility (%)
		at the start	at the and	Food (gr.)	Total nitrogen (mgr.)	in feces	in urine	Total		
I	May 30—Jun. 9 1924	97.0	111.0	23.08	193.50	25.15	103.58	128.73	+ 64.77	87.00
II	Jul. 14—20 1924	117.1	119.6	22.81	207.24	30.42	117.03	147.45	+ 59.79	85.32
V	Jul. 17—24 1924	109.6	116.4	24.50	279.30	19.04	119.51	138.55	+ 140.75	93.18
VI	Jul. 21—27 1924	108.9	119.1	21.38	217.27	22.45	111.63	134.08	+ 83.19	89.67

Both the rations A and B, studied here, permitted growth to almost the normal size. Group I on ration A, that contained 0.31% Na and 0.59% K in dry matter gained 57.0 gm. in weight per head for forty days, and Group II on ration B, that contained 0.41% Na and 1.03% K, gained 45.0 gm. but both the rations, that contained 0.92% Na and 0.35% K in the former and 0.76% Na and 1.15% K in the latter, did not maintain growth, and at last rats of Group III and IV died. The above trial began with the young rats, weighing from 35 to 56 gm. each.

When the second experiment was begun with rats weighing from 65 to 75 gm. each and the influence of the same rations on Groups III and IV was studied, each Group gained in weight 66.5 gm. and 67.8 gm. respectively per head for forty days as seen in the trial of Group V and VI in Chart I.

In this experiment it seems that the rations C and D are inadequate to the very young ones but do not show any injurious influence upon the one that grew already monerately on sufficient ration.

These results depend upon the age of the animal. During the earlier growing period the animal requires much more calcium and phosphorus than the alkali metals, with the result that an inadequate alkali ratio shows an adverse effect on the metabolism of calcium and phosphorus. The above discussion is derived from the comparison of growth in the most rapidly growing period.

The young animal, even once grown healthy on the complete ration, becomes deficient in inorganic matter after it is subjected to the ration devoid of salt. Such an animal cannot recover growth with the amount of inorganic nutrients contained in the ration C or D, although animals were fed on A or B recover growth. In such a state a loss in weight occurs as soon as ration C or D is given in place of A or B.

This fact is enough to constitute an unquestionable demonstration that the existence of adequate quantities of alkali metals in the ration is necessary for the maintenance of healthy nutrition of animal and at the same time a good ratio of alkali metals is also important. And it will be seen from the results of Groups VII, VIII and IX that rations C and D are not complete for all cases.

The extent of ratio of alkali metals in the ration used in this experiment was Na : K=1 : 0.375—2.505, which did not exercise any injurious influence upon the metabolism of nitrogen, and ration that contained a comparatively greater proportion of sodium rather increased the digestibility of nitrogen.

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ABSTRACTS FROM THE ORIGINAL PAPERS.

Concentration cells and Electrolysis of Sodium ethoxide solutions.

(*Ionic activity in alcoholic solutions*)

By MASUZO SHIKATA.

In order to prove the reversibility of the deposition of metallic ions in non-aqueous solutions, the deposition of sodium ions of sodium ethoxide in ethylalcohol has been tested.

In dilute solutions the deposition of sodium in alcohol proceeds quiet in the same manner as in aqueous solutions, i.e. has been proved to be reversible.

Through the application of sodium amalgam electrode, which has been specially designed for this experiment, in an aqueous as well as in an alcoholic solution as a reference electrode calculation of deposition potential of sodium in alcoholic solution has been attained.

Since the activities of sodium ions of more concentrated alcoholic solutions measured by concentration cells with sodium amalgam electrodes show an enormous increase of ionic activity, a number of concetration cell measurements have been carried out.

These abnormal ionic activities have been observed in aqueous solutions in cases of highly concentrated region, but far conspicuous are in concentrated alcoholic solutions; for example 2.891 normal sodium ethoxide solution shows discrepancy 0.073 volt from the calculated value by Nernst's formula. Remarks have been given as to diffusion potentials of these solutions.

Conductivities and vapour pressures of these solutions make one convinced of that this anomaly is due to the diminution of solvation of sodium ions.

Present author thinks it more reasonable to express the ionic activity of highly concentrated solution by the special term of solvation energy dH (and not by the ordinary activity coefficient "a," including the activitiy of ions both in dilute as well as in concentrated solutions.

That is $$E=\frac{RT}{nF}\ln\frac{a_1 C_1}{a_2 C_2}+dH$$

instead of $$E=\frac{RT}{nF}\ln\frac{a_1 C_1}{a_2 C_2}$$

A New Colour Reaction of Ketonic acids, Especially Laevulinic acid.

By Kanroku Kurono, Masaichi Yamada and Shō Ishida.

Since a remarkable discovery of carboxylase was made by C. Neuberg and his coworkers, ketonic acids have occupied an important position in the research of fermentation.

In such an investigation authors have made physiological experiments about laevulinic acid, and have been urged to find suitable reactions for the acid.

Several reagents previously mentioned as characteristics for laevulinic acid, such as Legal's reagent (sodium-nitroprusside with caustic soda), Rothera's (sodium-nitroprusside with ammonia and ammonium sulphate) and Bigwood's improved reagents, and also iodoform forming reagent are all common to other ketonic acids and ketones. Orcin-hydrochloric acid reagent for laevulinic acid as one of decomposition products of thymus nucleic acid used by Levene and Mandel is not so sharp, therefore the acid is hardly to be found about to 0.1 percent.

Quantitative such as that by reduction of silver nitrate or by oxidation to acetic acid, using sulphuric acid and bichromate as oxidizing agents, are not hindered by other organic acids when they are mixed.

As already known, Seliwanoff's reagent (resorcin hydrochloric acid) for fructose gives red colour by laevulinic acid and the author found Pinoff's reagent (ammonium-molybdate with acetic acid) suitable for fructose which also gives blue coloration by the acid and these two reagents are common to pyruvic acid.

A new reaction for laevulinic acid detected by the author is of a bluish green coloration with "vanilline sulphuric acid" reagent (1 g. of vanilline in 200 gs. of conc. H_2SO_4.), which was previously used by T. Takahashi & as reagent for fusel oil.

This reaction is the most characteristic, delicate (to 0.0019 percent) and accurate for laevulinic acid, though several alcohols, esters, acids, aldehydes and ketnones also produce individual colorations. Pyruvic acid likewise gives the same but differentiating from laevulinic acid in its bluer predominant coloration, and its sharpness. The pyruvic acid which has the strength of 0.1% gives faint olive green or brown tint when coming in contact with the acid and the reagent.

As pyruvic acid and laevulinic acid are both ketonic acid, the reagent may be used as ketonic acid reagent qualitatively and also quantitatively by colorimetric method.

But this colorimetric method can not be used directly on the substances such as fermentation liquid; because sugars, lactic acid, fusel oil, esters and other ingredients prevent the reaction indicating miscellaneous colorations.

Then treat as follows:-

Evaporate the fermentation liquid in the water-bath with repeated addition of distilled water to remove alcohols, esters and other volatile substances. After cooling residual liquid is extracted with ether four or five times after which evaporate the extract in order to expel the ether. The residue is dissolved in water and neutralized with lime water. This is again evaporated in the water-bath and the residue is crushed and extracted with absolute alcohol in the water-bath under reflux condenser for about half an hour. The calcium salt of laevulinic acid dissolves out to alcohol but calcium lactate does not. After evaporation of alcoholic extract, residue is extracted with ether several times to remove fusel oil and other substances. Here the remaining calcium laevulinate is filled up to a definite volume and is provided for quantitative test.

The reagent is the most suitable if 2 c.c. is added to 1 c.c. of sample, and the maximum coloration appears at about fifteen minutes after the addition.

Sakē or "Shōyu" (Soya sauce) does not contain laevulinic acid. We found in our other experiments that Sakē yeast or Shōyu yeast could easily decompose the acid.

On the Influence of Various Ratios of Sodium and Potassium upon the Growth of Animals.

By RINJIRO SASAKI.

(*From the Agricultural chemical Labolatory, Department of Agriculture, Tokyo Imperial University.*)

N. Zuntz(1) in an early study cited the comparisons of the ash of normal hays with those of hays causing disease, and proving that growth and nutrition of the farm animals were seriously affected by the alkali ratio in the ash of hay and showing, from the results of analysis, the alkali ratios of normal hays to be $Na_2O : K_2O = 1 : 3.68—4.75$, while those of injurious hays were 1 : 21.64—178.10, and stating that the injuriousness or non-injuriousness for nutrition of the farm animals was due to the alkali ratio of the ash.

M. B. Richards, W. Godden and A. D. Husband(2) in the study of metabolism of growing pigs reported that the addition of sodium chloride or citrate to the fodder, consisting of grain mixture, increased the assimilation and retention of nitrogen, calcium and phosphrus in the body, at the same time even after the fodder, with the addition of sodium salt, is fed for two weeks thereby increasing the excretion of potassium in urine and decreasing that in the feces. From the above results it seems that a close relation exists between sodium in the fodder and the metabolism of potassium.

I(3) have already shown that both sodium and potassium were indispensable for the growth of the young animal and could not be replaced, from the results of feeding experiments on white rats. And I(4) have also previously shown that when the content of sodium was insufficient in the diet, the digestibility of nitrogen became bad and that the excretion of sodium in feces became parallel to the digestibility of nitrogen and that the balance of nitrogen also became parallel to that of potassium. In this experiment the alkali ratios of the ash of diets were $Na : K = 1 : 1.54$ and 1 : 6. 67. I have, therefore, continued the investigation in order to discover whether absolute quantity of the two alkali metals contained in diet or their ratio have an important influence upon the growth of young animals.

EXPERIMENTAL

The four rations used in the experiment described in this paper consisted of purified casein, potatoe starch, lard and salt mixture (sodium chloride, potassium chloride, magnesium sulphate, disodium phosphate, dipotassium phosphate, monocalcium phosphate, calcium lactate, ferrous lactate), and all the ingredients existing in sufficient quantities of which only the ratio of the alkali metals was variously changed. Besides the above nutrients, cod-liver oil and commercial oryzanin alcoholic solution were added in adequate quantity for the source of vitamin A and vitamin B.

The synthetic ration consisted of the above nutrients as casein 18, starch 70, lard 6, salt mixture 4, and cod-liver oil 2%. The contents of the alkali metals in the four synthetic rations are given in Table I.

TABLE I.

Contents of Alkali metals in Rations.

Rations	In Dry Matter.		Ratio.
	Sodium %	Potassium %	Na : K = 1 :
A	0.3067	0.5884	1.918
B	0.4094	1.0255	2.505
C	0.9237	0.3465	0.375
D	0.7558	1.1454	1.515

The rations were mixed with distilled water and made into a firm, pasty mass by stirring them in the boiling water bath to prevent scattering as far as possible. The animals were experimented giving free access to distilled water and to the desired ration. The feeding cage was equipped with a false bottom with wire screen to obviate as far as possible the consumption of excrete.

In the first experiment the comparison began with eight litters of white rats weighing from 35—56 gm. each; they were divided into four groups each of them consisting of two rats. The rations used are given on Chart I illustrating the growth curves.

In the second experiment four young rats, weighing from 65—75 gm. each, were divided into two groups, one of which cotains two rats, and the influences of the ration C and D were observed.

In the third experiment, seven litters of group II, born in the first experimental period, weighing from 54—75 gm. each, were divided into three groups. The rations used are given on Chart II illustrating the growth curves.

(1) Growth.

It will be seen from Chart I that the growth of rats on Rations A and B were almost normal but rats on Rations C and B could not grow and died, after having lived for from 15—50 days from the beginning of the experiment.

In the second experiment Groups V and VI, each on Rations C and D, grew almost the same as on Rations A and B in the first experiment in Chart I, so it seems that they had already reserved enough inorganic nutrients in the body before the experiment.

In the third experiment, when young rats were placed upon the synthetic rations devoid of salt mixture for thirty days, they stopped growing but did not decrease body weight, although five days later they lost rapidly in weight and it seemed that they became deficient in inorganic nutrients to maintain health.

As seen in Chart II, rats on rations B, C and D could not recover thire growth and Groups VII and X could not gain anything and died even after having received Ration A. Group VIII, fed on ration A, gained in weight after forty days, but on ration C again decreased on the fiftieth day, and five days later on Ratian D decreased more and on ration B again gained.

From these trials it appears that Rations A and B are adequate for supporting growth, although rations C and D are not satisfactory for the very young rats less than from 35—56 gm. in weight.

In the last trial, there were a few animals that could not recovered body weight after decreasing on the ration which as entirely devoid of salt mixture for thirty days. It is doubtful whether they exhausted the alkali neutralizing acid which was produced by the decomposition of substances in the body.

To obtain the daily intake of alkali metals, residues remaining in the food cups were removed each day, dried and subtracted from the ration offered.

The results and calculations pertaining to the first, record, fifth and sixth groups of eight rats are given in Table II.

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ABSTRACTS FROM THE ORIGINAL PAPERS.

Studies On the Amylases of Different Origins for Alcohol Brewing I.

Quantitative Comparision of Amylo-liquefying, Amylo-dextrinizing and Amylo-saccharifying Enzymes of Germinated and Ungerminated Cereals, and Molds.

By KOKICHI OSHIMA and SHINICHI ITAYA.

The purpose of this study is to ascertain the best amylolytic agent and condition for alcohol brewing by comparing many kinds of amylolytic enzymes, especially of molds, such as Aspergillus oryzae and Asp. awamori, which have special use in Japanese breweries. Also the effect of mixing of mold amylase and malt amylase as the saccharifying agent.

A. Enzymic samples experimented were as follows:—

a. Purified enzymes (mostly alcohol precipitant) of germinated barley, soya beans, Aspergillus oryzae and Asp. awamori.

b. Molds—Asp. oryzae, Cohn (3 strains), Asp. awamori, Nakazawa, Rhyzopus delemar (Boid) Wehm. et Hanz. and Rh. japonicus Saito. These were cultured on cooked wheat bran, which was considered the best medium to produce the strongest amylase. The enzymic activities were tested with the water extract.

c. Germinated and ungerminated cereals—barley (Hordeum sativum, Jensen) (3 kinds), wheat (Triticum vulgare, Vill), rice (Oryza sativa, L.) (2 kinds), oats (Avena sativa, L.) (2 kinds), rye (Secale cereale, L.), soya beans (Glycine soya, Benth), barnyard grass (38 kinds) (Panicum Crus-Galli, L.)

B. Experimental methods:—

a. Amylo-liquefying enzyme. With Osima-Itaya's method (11), by the change of viscosity of starch paste, digested at 40°C for 30 minutes.

b. Amylo-dextrinizing enzyme. With a little modification of Wohlgemuth's method (18), by iodine reaction of soluble starch solution, digested at 40°C for 30 minutes.

c. Amylo-saccharifying enzyme. With Oshima's method (9), by reducing power of produced sugar from digested soluble starch solution at 40°C fot 30 minutes.

All enzymic activities (=quantities) were measured for the unit of one gram of original dry cereal or dry culture medium.

C. Optimum reactions and quantitative estimations of the three kinds of amylases of the samples. The amylolytic activities were tested in every sample but only the strongest sample of each species is here described.

Samples	Amylo liquefying enzyme		Amylo dextrinizing enzyme		Amylo-saccharifying enzyme	
	optimum P_H	quantity	optimum P_H	quantity	optimum P_H	quantity
Purified enzyme of Asp oryzae	4.5-5.2	36,000	5.2	50,000	5.2	4,040
〃 germ. barley	4.5	20,000	4.5	33,340	4.5 6.2	6,100
〃 ungerm soya beans	5.2	800	5 2	260	4.8	21,000
〃 Asp awamori	4.5	10,800	4 5 5.2	20,000	5.2	2,740
Asp. oryzae	4.5	1,000	4.5	833	5 2-5.8	206
Asp. awamori	4.5	388	4.5	250	4.8-5.2	55
Rhyzopus japonicus	5.2	400	5.2	250	5.2	194
〃 delemar	4.8	50	4.8	67	4.8	11
Germinated barley	5.2	125	5 2	555	4.5-4.8	166
〃 barn yard grass	4.5-5.2	400	5.2	500	4.5-5.2	41
〃 wheat	6 5	360	—	—	5.2-5 8	576
〃 oats	5.2 6.5	160	5.2	500	5 2	41
〃 rice	—	—	—	—	5 2	6
Ungerminated rye	4.5	13	4.5	55	4.5	132
〃 rice	—	—	—	—	5 2	0.1
〃 barn yard grass	—	—	—	—	—	0
〃 soya beans	—	—	—	—	4.8	295
〃 barley	—	—	—	—	—	0
〃 wheat	6.5	1.4	—	—	5.2	118
〃 oats	—	—	—	—	—	0

D. Conclusion and discussion.

1. The optimum reaction of the actions of three amylases from various samples

were almost same, i. e., PH 4.5–5.8. Only exception was wheat (ungerminated), which had PH 5.2–5.8 for amylo-saccharase, and PH 6.5 for amylo-liquefying enzyme.

2. Reaction range of the considerable activity was in general PH 3.0–7.0, as exception wheat amylase acted at basic side a little more.

3. The amylo-liquefying and amylo-dextrinizing enzymes variated with almost same ratio for every sample. On the contrary, the amylo-saccharifying activity variated independently with the two above.

4. To compare the amylolytic activities of different samples, purified enzymes must be omitted, as they differ according to their preparation method. The strongest amylo-liquefying enzyme was of Asp. oryzae, the next was germinated barn yard grass, Asp. awamori, germinated wheat. The strongest amylo-saccharifying enzyme was in the order of Asp. oryzae, germinated barley and ungerminated rye.

5. Ungerminated soya beans, germinated wheat, germinated barley and ungerminated rye had stronger amylo-saccharifying than amylo-liquefying enzyme comparing with Asp. oryzae, Asp. awamori, Rh. delemar and Rh. japonicus. On the contrary, the last group had stronger amylo-liquefying enzyme than amylo-saccharifying enzyme. What would be the effect of mixing the two groups? This will be presented in next report.

6. The comparison some kinds of germinated barley showed the amylo-saccharifying activities between 146–340. The length of the cotyledon over 1 cm. had little change on enzymic activities.

7. 38 kinds of germinated barn yard grass showed the amylo-saccharifying activities between 6.4–40.8 and the average was 16.5.

8. Comparison between glutinous and common rice showed not much difference for their amylo-saccharifying activity. Both activities increased about ten times by germination. Still the strongest activity was only 6.0.

9. Ungerminated rice, barley, oats and barn yard grass had little amylase, but ungerminated soya beans, wheat and rye had quite strong amylase. By germination, wheat increased its amylo-saccharifying activity 5 times, whereas its amylo-liquefying activity 250 times.

10. Oats of several kinds showed similarly the saccharifying activity about one fourth of that of germinated barley, but the liquefying activity was stronger than the latter.

11. Three strains of Asp. oryzae were compared their optimum reaction and activity of 3 amylases. The optimum reaction for three amylases remained the same. The ratio of the activity of three amylases was almost same and they showed much stronger amylo-liquefying activity than germinated cereals. The amylo-saccharifying activities were 68–206. Other experiments (10) showed that the activity of many strains of Asp. oryzae variated greatly. The strongest amylo-saccharifying activity was 260, which is probably the strongest amylase produced among molds.

On Sulphur Containing Amino-acids. IV.

The Determination of Cystine in Urine.

By Yuzuru Okuda and Yuraku Nishijima.

From the Biochemical Labolatory, Department of Agriculture, Kyushu Imperial University, Fukuoka, Japan.

A new method for the determination of cystine in urine is described. It is an application of the iodine method recently published by one of the authors (Okuda: J. Chem. Soc. Japan, **45**, 18, 1924.······Okuda and Motomura: This jounal, in press).

The method is directly applicable in the normal urine and also in the presence of sugar, but indirectly in the presence of much protein and hydrogen sulphide. Most of the protein should be previously removed by boiling with a few drops of acetic acid and hydrogen sulphide by aeration.

The Method:—The following solutions are required,···(1) About 5% KI aqueous solusion. (2) Exactly 4% HCl. (3) Exactly 2% HCl. (4) Exactly 20% NaOH. (5) Cystine solution, which contains 1 mg, cystine in 1 c.c. of 2% HCl. (6), M/1000 KIO_3, which is prepared by dissolving 0.214 g. of pure KIO_3 in 1 liter of exactly 2% HCl. For standardization of this solutson for cystine, dissolve 0.2 g of cystine in 50 c.c. of about 5% HCl, add a few decigrams of zinc dust, leave it for 30 minutes at room temperature. Filter, wash and make it up to 100 c.c. Take 1 c.c. of the filtrate in a dry bottle or a test tube, mix with 19 c.c. of exactly 2% HCl, 5 c.c. of 5% KI and 5 c.c. of exactly 4% HCl, and then titrate with the iodate solution until a yellow colour is produced. Insert immediately a thermometer in the mixed liquid to know the temperature in which the experiment has been finished. The quantity of the iodate solution corresponds to 2 mg. of cystine, at that temperature. It is convenient to repeat the same experiments several times in different temperatures, and to get a table or a curve showing the relation between the temperature and the required volume of the iodate solution.

Procedure :—Take 50 c.c. of urine, add 10 c.c. of the cystine solution, boil for 1 minute with about 0.5 g. of the best charcoal and then cool for 10 minutes. Filter with a small Buchner funnel, wash thrice with 5 c.c. of water. Add about 0.5 g. of fine zinc dust and 15 c.c. of about 20 % HCl. Leave it for 30 minutes at room temperature for reduction. Filter again with a small Buchner funnel, wash twice with 5 c.c. of water. Make the filtrate up to 100 c.c. with water. Take 1 c.c. of the solution for the determination of the concentration of HCl in it. To 90 c.c. of the residual portion of the solution, add a calculated quantity of 20% NaOH to make it into a solution containing exactly 2 % of free HCl. After ascertaining by titration that the solution contains exactly 2 % HCl, take 20 c.c. of the solution, mix with 5 c.c. of 5 % KI and 5 c.c. of 4% HCl, and then titrate with the standard iodate solution until the yellow color produced remains for 1 minute. Read the temperature of the liquid. Calculate the amount of cystine in the 20 c.c. of the solution, using the table or the curve previously obtained. Subtract 10 mg. from the total amount (mg) of cystine obtained, then the rest is the quantity of cystine in 50 c.c. of the original urine.

If the original sample contains some cysteine, the results obtained as above express the sum of cystine and cysteine as cystine.

The separate determination of cystine and cysteine is easily accomplished, titrating a sample solution before and after the reduction.

It the special case of urine which contains too much thiocyanate, this should be removed. The original paper describes a method to deal with such a case. But the case is very rare.

Looney's method (Jour. Biol. Chem., **54**, 171, 1922.) for the determination of cystine in urine was compared with the present method. The former gave much higher results, especially in the case where less uric acid is present in the urine.

On the Waxy Substance Coating on the Cuticle of the Bamboo Joints.

K. KAWAKAMI.

Phyllostachys, Edulis A., et, C. Riviere, the largest stem species of the bamboo grow in Japan, has a white powder-like waxy substance on the cuticle of its joints. This substance is scraped off from the bamboo nodes with finger-tips and tested. It melts at 80°-81°C., and does not dissolve in ethyl alcohol, methyl alcohol, carbon tetrachloride, and ether at the room temperature, and also scarcely soluble even being heated, however, somewhat soluble in chloroform and carbon disulphide at the ordinary temperature, and more readily when heated It does not give the Liebermann's cholesterol reaction like those waxes of grape or apple.

The most part of the soluble substance in cold chloroform consists of a higher paraffin, which being isolated in a pure state by distillation at a diminished pressure. The substance thus obtained seems to be a hydrocarbon of methane series, having a melting point of 63°-64°C, being identical with that of nonacosane; and by its analysis and the molecular weight the substance is found to be very likely same as nonacosane:

Analysis		found	(1)	C = 85 12%	H = 14.67%
			(2)	C = 84 65″	H = 14.65″
Nonacosane	$C_{9}H$	Calc.		C = 85 29″	H = 14.71″
Molecular weight		found	(1)	395	
			(2)	385	
	$C_{29}H_{60}$	Calc.		408	

The insoluble part of the wax in chloroform at room temperature is saponified with 5% Na-alcoholate, and $CaCl_2$ is added as to make a Ca-soap. Then the alcohol is evaporated off, and the residue is extracted with hot chloroform in oder to remove the wax-alcohol and hydrocarbon present. After the resulted substance is decomposed by boiling with dilute HCl, a certain fatty acid is formed, and it is decolorized, and recrystallized from ethyl alcohol, and finally is crystallized in a fine needle form. Its melting point is 78°C., which is almost identical with that of melissic acid, which melts at 78.5°-79°C. The result of analysis, and the molecular weight are as follows.

Analysis :	found	(1)	C = 79.06%	H = 13.20%
		(2)	C = 79.76 "	H = 13.14 "
Mellissic acid $C_3 H_6 O_2$	Calc.		C = 79.65 "	H = 13.27 "
Molecular weight	found	(1)	432	
		(2)	453	
$C_3 H_6 O_2$	Calc.		453	

The above extracted portion of the saponified substance is separated into the two parts:—the wax alcohol and the hydrocarbon, by the method of Leys, (J. Pharm. et chim. **5**, 277, 1912), and the part of wax alcohol is several times recrystallized from ethyl alcohol, and finally is made in a needle crystalline form, which has a silky lustre, melting at 85°–87°C. The author believes this substance should be melissyl alcohol, which melts at 86°–88°C. The figures of the analysis, and determination of the molecular weight are shown below.

Analysis :	found	C = 82.19%	H = 13.97%
Melissyl alcohol $C_3 H_{61}OH$	Calc	C = 82.19 "	H = 14.16 "

The result of analysis of the acetate of the alcohol is as following.

Analysis :	found	C = 80.02%	H = 13.57%
$C_3 H_{61}COOCH_3$	Calc.	C = 80.00 "	H = 13.33 "

Besides those three compounds, the portion that is soluble in cold chloroform contains a crystalline substance which can be dissolved in acetic acid, and so it is obtained by extracting it with 80 % acetic acid. When the extract is concentrated to about half its volume the substance is crystallized out itself. It is soluble in chloroform, hot ethyl alcohol, and acetic acid. It contains a certain mineral matter, and melts at 240°–250°C with decomposition. The yield of the substance is so poor that the author could not study further more.

The mother liquid contains some resin which is soluble in most organic solvents and can be burnt readily giving a characteristic smell resembling that of terpentine.

Quantities of those substances were as follows.

Nonacosane	$C_{29}H_{60}$	55%
Melissic acid and Melissyl alcohol ($C_3 H_6 O_2$ + $C_3 H_{61}OH$)		20 "
unknown crystalline substance		2 "
resin		4 "

On Vitamine C in Japanese Sand Pear (Pyrus serotina Rehder) and Kaki-Fruits (Diospyros Kaki L.)

By YASUO IWASAKI.

The author found that the vitamine C content in the flesh of Japanese sand pear, Chōjuro (a horticultural variety), was very poor, but when its rind and flesh were compared, the rind part was a little richer.

The method used by the author for the experimental scurvy was the following: growing guinea pigs were fed on the mixture of equal volume of wheat bran and rolled oats at libitum, a daily ration of 40 c.c. of milk autoclaved for an hour at 120°C. and water given freely after the animals had taken the milk. After the animals had shown obviously the scorbutic signs, several amounts of the juice of the above named fruits to be tested were given to them in addition to the above foods. The juice of each fruit was freshly prepared every day before giving it, being grated and squeezed the fruit through cotton-cloth.

45 c.c. of the juice of pears, not containing the rind part were added every day, but this could not cure the scurvy, and the animal died on the 26th day from the time the juice had been added.

The juice from the flesh containing rind part showed better antiscorbutic potency, because the animals which took 41 c.c. of it every day, became gradually cured from the disease, and after 40 days they restored their health utterly. One animal which took 34 c.c. of the juice every day, showed a slight symptom of scurvy yet.

The juice of the ripe Kaki-fruits Fuyu (a horticultural variety) was given to two animals, 10 c.c. per day for 30 days. The animals cured completely during the period. So it can be said that the Kaki juice has a very good antiscorbutic power, but its minimum dose which can cure scurvy can not be determined from these experiments.

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ABSTRACTS FROM THE ORIGINAL PAPERS.

The Electrolysis of Nitrobenzol at the Mercury dropping cathode

Part I. The reduction potential of nitrobenzol.

By MASUZO SHIKATA.

The dropping mercury cathode has been found to give exact deposition potentials of metallic ions from their aqueous as well as alcoholic solutions.

It is naturally of interest to see how far this method can be applied for the study of cathodic reduction of organic substances.

Nitrobenzol has been chosen as one of the typical organic electrolytic reductions hitherto systematically studied.

Series of experiments have been carried out in acidic, neutral and alkaline solutions.

In acidic as well as neutral media the relation between reduction potential and system nitrobenzol–nitrosobenzol–hydrogenions follows approximately to Nernst formula.

The reduction in acidic solution is, of course, due to the deposition of hydrogen ions, and in neutral solution due to the ionic splitting of water molecules.

In alkaline solution, however, is quite contrary to the value expected from Nernst formula, i. e. in 0.1 n NaOH deviation of +0.176 V, in 1 n NaOH+0.365 to positive, which is quite unexplicable from the inorganic electrolytic reduction.

Three possible explanations have been proposed and discussed.

(1) Pseudoacid reaction. (Hantzsch's Pseudosäure)

(2) Direct electronic reduction.

(3) The retardation of reduction in acid media and normal reduction potential in alkaline media.

An explanation of reduction by assuming temporary deposition of metallic ions, which had been hitherto taken as possible, has been disproved by showing that electropositive metal such as zinc and lead have no influence upon reduction potentials of nirobenzol.

The effect of neutral salt upon reduction potential has been proved to be the salting out action of neutral salt.

The following general formula has been given, exclusive of alkaline solutions.

$$\pi = -\frac{RT}{2F} \ln \frac{k}{C_{RNO_2}^{\frac{1}{m}} [H^{\bullet}]^2}$$

where $\frac{1}{m}$ is adsorption exponent.

For the same hydrion concentrations

$$\pi = -k'' + \frac{RT}{2mF} \ln C_{RNO_2}$$

where $m = 1.26$ in acidic, $m = 1.31$ in neutral and $m = 2.2$ in alkaline solutions, showing higher adsorption in alkaline solution.

Part II. The influence of the cathodic potential on the adsorption of nitrobenzol.

(*The electro-potential adsorption of nitrobenzol*)

The polarization curves observed at the mercury dropping cathode show very often a distinct maximum at which, the current after having reached a certain intensity, begins to decrease with the increase of voltage, which is never the case in inorganic electrolysis.

To investigate this phenomenon more closely, a number of experiments were carried out.

To give a clearer idea of meaning of current-voltage curve, a comparison has been made between non polarizable electrode and mercury electrode.

Two kinds of saturation curves are observable, one due to a lack of hydrogen ions and the other due to that of nitrobenzol.

The maximum current is observable in the case of nitrobenzol saturation curve.

It has been found that nitrobenzol has an influence upon the absolute zero potential of mercury drops, which is always characterized by the minimum oscillations of galvanometer.

The high adsorbability of nitrobenzol to zinc is known and the same can be said to mercury.

Taking the view of Langmuir, we can assume that nitrobenzol is adsorbed to mercury, arranging oxygen atom to the mercury surface.

From this stand point it is not unnatural to assume that the adsorption of nitrobenzol is due to electrostatical attraction between oxygen atom of nitrobenzol and mercury, the latter, of course, is much influenced by the polarization potential applied to mercury drops.

In treating the effective concentration (C) of nitrobenzol, it is much reasonable to take the concentration at the adsorptive layer, that is

$$C=k'(E-E_0)-k''[H^\bullet]+C_0$$

where E is the applid e. m. f. E_0 the absolute zero potential in the presence of nitrobenzol, C_0 the bulk concentration, $[H^\bullet]$ the hydrion concentration.

From these view points the present auther deduced a mathematical formula

$$E=-\frac{RT}{2F}\ln\frac{kI}{[H^\bullet]^2(k'(E-E_0)-k''[H^\bullet]+C_0)}$$

where E the applied e. m. f. and I the corresponding current intensity.

It follows

$$I=\frac{[H^\bullet]^2(k'(E-E_0)-k''[H^\bullet]+C_0)}{k}e^{-\frac{2EF}{RT}}$$

For the maximum current intensity i. e. for $\frac{dI}{dE}=0$

we have $E-E_0=\frac{RT}{2F}+\frac{k''[H^\bullet]}{k'}-\frac{C_0}{k'}$

In alkaline solution, where we can neglect the term $\frac{k''[H^\bullet]}{k'}$, $(E-E_0)$ must theoretically always smaller than $\frac{RT}{2F}=0.0126\,V$.

Difference between potential of maximum current and absolute zero potential has been experimentally found to be 0.0115 V which is, in fine accord with the theoretical value.

For higher hydrion concentration $(E-E_0)$ will become larger which is also the case in experiments.

Further the influence of neutral salt, i. e. its salting out action, has been determined numerically, e. g. for 0.1n NaCl 6.7% and for 1 n NaCl 60% of the bulk concentration of nitrobenzol present.

The possibility of applying this method for the determination of nitrobenzol has been proposed. (the concentration of nitrobenzol so far as 10^{-5} gr. mol in litre gives saturation wave.)

Attention must be drawn to the new factor in the organic electrolytic reduction of technical process in pursuing the maximum current efficiency, that is, the electro-potential adsorption of organic substance to the electrode.

On the Enzymic Actions of Malt Diastase, purified fractionally by Ethyl Alcohol, upon the various Kinds of Starch and Soluble Starch.

By Fumiwo Hemmi and Mitsuji Ito.

I. Purification of Malt Diastase by Ethyl Alcohol with Different concentrations.

The kilned malt for beer brewing was extracted with 20 % alcohol (vol. %). To the filtrate, strong alcohol was added making it altogether 71.5 %, 77.3 %, and 82.8 % in volume successively. From these three fractions of malt diastase were precipitated. The first fraction, precipitated from 71.5 % alcoholic solution, was gathered on a filter paper. Then the alcoholic content of the filtrate was increased to 77.3 % by a further addition of strong alcohol. From the filtrate separated from the second precipitate, the third fraction of enzyme was precipitated by 82.8% alcoholic solution. The three fractions of the enzyme thus obtained were well washed first with alcoholic solution of such concentration as precipitated the enzyme, then with absolute alcohol and ether several times, and afterward dried. Each fraction was dissolved in a definite quantity of water in regulating the concentration of enzyme.

II. Preparation and Purification of various Kinds of Starch and Soluble Starch.

Starch used in the present investigation was made and purified from the following eleven kinds of plant:

1. Erythrinum denscanis L.
2. Manihot aipi Pohl. (glutinous).
3. Maranta arundinacea L.

4. Oryza sativa L. (common).
5. Oryza sativa L. (common, up land).
6. Oryza sativa L. (glutinous).
7. Panicum frumentaceum Roxb.
8. Panicum miliaceum L. (glutinous).
9. Setaria italica Kth. var. germanica Trin. (glutinous).
10. Solanum tuberosum L.
11. Zea mays L.

Soluble starch was made from pure starch above prepared. Fifty grams of pure starch were put in a flask, containing 200 c.c. of dilute hydrochloric acid (1 vol. of 35.39% hydrochloric acid: 9 vols. of water) and kept at room temperature, well shaken twice a day. Fifteen days after, the upper solution was removed dy decantation. The soluble starch thus obtained was well washed with water for a long time, until no acid reaction was observed; then with alcohol and ether, and afterward dried. The starch paste and the soluble starch solution thus obtained were all neutral in reaction.

III. Experiments for Enzymic Actions of Malt Diastase.

The experiments for the liquefying and the sacchrifying powers of malt diastase were made separately, using different fractions of malt diastase, and various kinds of starch and soluble starch. 2 % and 4 % starch pastes, and 2 % and 5 % soluble starch solutions were used. The experiments were made at four stages of 2.5 or 4.5, 20 or 25, 45 or 50 and 100 hours respectively. Incubation, at 38°C. Toluene was added for antiseptic purpose as well as to prevent the evaporation of water during the incubation. The relative power of liquefaction of starch paste by malt diastase was determined by using Ostwald's viscosimeter and by color reaction with potassium iodide iodine solution. The relative power of saccharification of starch paste and soluble starch solution was measured by the Bertrand's method and estimated by volumes in c.c. of $KMnO_4$ solution used for titration.

IV. Results.

1. When the malt diastase was purified fractionally by ethyl alcohol with different concentrations, enzymic powers of each fraction of malt diastase were different. The relative powers of liquefaction and saccharification of the first and the second frations of the enzyme were both strong, especially the former was the strongest of three. The third fraction of malt diastase had weak powers of these

two enzymic actions and specially for potato starch and its soluble starch.

2. Both actions of lipuefaction and saochrification of malt diastase upon various kinds of starch and soluble starch were different, according to the special properties of starch and soluble starch and to fractions of enzyme and also to their concentration.

3. The relative power of saccharification of malt diastase upon various kinds of starch was lower than that upon soluble starch, in every fraction of three, with the exception of potato starch and its soluble starch. Further investigations on the enzymic action of malt diastase upon potato starch and its soluble starch will be reported in a subsequent paper.

Feb. 28 th., 1925.

Feeding Experiments on the Nutrition of Chickens and Fowls.

By Masumi Kanai and Masanobu Matsuda.

The influences of vitamins and some kinds of proteins on the nutrition of chickens and fowls, and on their egg-production have been investigated as follows ;-

1. Vitamins A and B are absolutely necessary for the growth of chickens. In case the diet lacks of them the growth stops and death is followed. This influence is more remarkable on the younger ones, and the effect of vitamin B seemes to be more rapidly noticed than that of A.

2. When chickens are fed with an amount of vitamin A and B in more than the necessary quantity, their bodily resistance becomes much greater as the additional amount is increased. Vitamin A is exceedingly more effective on chickens in this respect.

3. Vitamin C is also necessary for their growth, but its influence is not so great as that of A and B. The supply of this vitamin can be discontinued, after its necessary quantity is given, without any interference in the growth.

4, The effects of vitamins A and B on fowls are similar to those effects on chickens. When these vitamins are not supplied, the fowls cease to grow further, and their body-weights decrease. But fowls show a greater resisting power to the deficiency of A and B than do chickens. Vitamin B is more effective on fowls than vitamin A, and shows identical influences with those on chickens.

5. Vitamin C is not necessary for the growth of fowls. With regard to egg-production the authors have to make another investigation.

6. The addition of vitamins A and B to the usual ration increase the egg-production. This influence is more pronounced in the case of A than of B.

7. As to the protein supply for the growth of chickens, pure proteins alone are not sufficient; but a certain quantity of soluble proteins must be added.

8. Fresh vegetable proteins have almost the same nutritive value as horse-fresh proteins for the growth of fowls, and when fresh proteins are given as a part of the indispensable food ration, the only other necessary proteins are those of fresh vegetables. (Chickens partly want some soluble proteins). Under these conditions they grow without showing any defect and increase greatly in their body weights.

Concernig the difference between the influences of fresh vegetable proteins and fresh proteins on the growth of fowls, the authors will discuss in another paper.

On the Occurrence of a Sulphur-containing Amino acid in Yeast.

By Satoru Odake.

Last year, Dr. U. Suzuki, T. Mori and the author isolated a new sulphur compound from the alcoholic extract of yeast, and gave the emperical formula (C_{11} H_{15} NSO_3 to it. Boiled with diluted acids, it was hydrolysed easily to adenin $C_5H_5N_5$) and a new thiosugar ($C_6H_{12}SO_4$); so the authors concluded this compound should be Adenyl-thiomethyl-pentose (U. Suzuki, S. Odake and T. mori; - The Journ. of the Agricul. Chem. Society of Japan, Vol. I, No. 2, p. 127-136, 1924, Biochemische Zeitschrift, B. 154, Heft, 3/6 S. 278-289, 1924.)

On studying further the alcoholic extract of yeast, the author isolated a sulphu containing amino acid in the following way:—

The alcoholic extract of yeast was evaporated in diminished pressure to a syrupy consistence and dissolved in a little water. A concentrated tannin solution was then added and the precipitate thus formed, was collected, decomposed with baryta water and filtered. The filtrate, freed from an excess of baryta, was evaporated to a small volume, when the crystals of adenyl thio methyl pentose separated out and which were filtered off. To this filtrate, strong alcohol was added enongh to make the alcoholic content of the mixture 80% by volume. The voluminous precipitate thus formed, was filtered by suction, and recystallized several times from diluted alcohol. The crystals were found to be the mixture of leucin and a sulphur compound, but it was impossible to separate them by fractional cryrtallization. It was therefore dissolved again in water, and the saturated solution of mercuric chloride was added. The sulphur compound alone, forming an insoluble double salt with it, was precipitated and then decomposed with hydrogen sulphide. This treatment was repeated again and the resulted crystals were recrystallized from diluted alcohol. The yield of the purified compound was 0.6 gr. from about 20 tons of fresh yeast.

The sulphur compound thus obtained having the emperical formula $C_5H_{11}SNO_2$ is apparently a sulphur containing amino acid, as the analytical results of the free compound as well as of its derivatives show.

The purified compound is colourless and crystallizes in thin monoclinic plates. Heated in a capillary, it melts at 272–273°C (uncorr.) with decomposition. It is easily soluble in water, and in diluted alcohol, but insoluble in ether, benzene, e t c. Its specific rotatory power is $[\alpha]_D^{16} = -11.77°$ in water. The aqueous solution of this compound gives a violet colour reaction with ninhydrin when warmed, but Millon's, diazo–, biuret–, and ferric chloride reactions are all absent. With $HgCl_2$ and $HgSO_4$, it gives a white precipitate, but it is precipitated neither by phosphotungstic acid nor by picric acid. Even a boiling strong alkali does not split sulphur from this compound in a form, detectable by sodium nitroprusside or by lead acetate, while both these reactions are positive, when it is fused with metallic sodium. In contrary to ethyl cystein this sulphur compound is quite stable against a boiling strong alkali, giving neither ammonia nor ethyl mercaptane. (Compare ; Brenzinger:– Zeitschrift. f. physiol chem. XVI. 563, 1892. Neuberg U. Mayer :– Zeitschr. f. physiol. chem. 44. 489, 1905). The copper salt $Cu\ (C_5H_{10}SNO_2)_2$ forms light blue thin monoclinic plates which are somewhat soluble in boiling water but insoluble in the cold. Its derivative of α–naphtyl isocyanate $C_{10}H_7NHCO\text{-}NHCH\ (C_3H_7S)\ COOH$ crystallizes in white long needles, melting at 178°C (uncorr). It is almost insoluble in water, ether e t c., but dissolves easily in alcohol.

Recently, J. H. Müller isolated a new sulphur compound $C_5H_{11}SNO_2$ from hydrolytic products of casein and eggalbumin with snlphuric acid or caustic soda.

(J. H. Müller :- Journ. of Bact. VII. 300–325, 1922. Journ. of Biolog. chem. LVI. No 1. 1923.) The sulphur compound, isolated by the author from yeast, has entirely the same properties with it, except a little difference in the rotatory power. Although the constitution and the distribution of this compound are now in the course of investigation, it is clear that it must have been produced by autolysis of of yeast protein.

The author desires to express his sincere thanks to prof. Dr. U. Suzuki, for kind advice and giving the opportunity of publishing this paper.

Sinomenine and Dehydrosinomenine.

Part IV.

By Kakuji Goto.

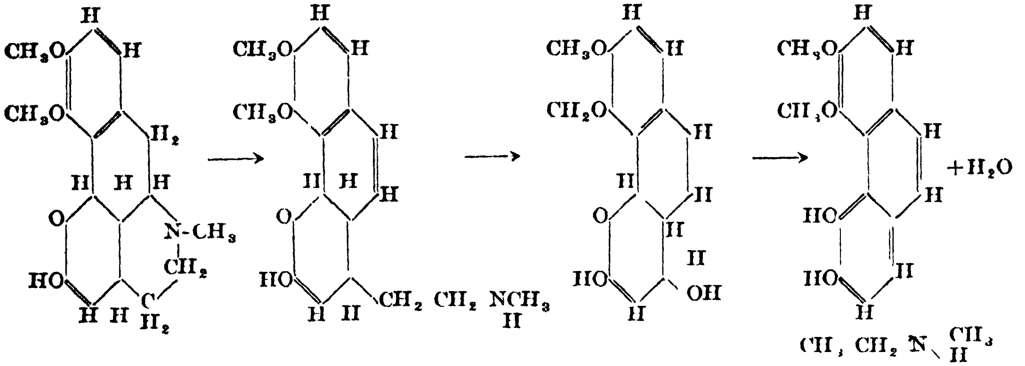
In the last report (this Journal, this vol., p. 50), it could not be decided whether the amine, obtained from sinomenine by the fusion with kali was methylvinylamine or methylethylamine. For, the melting point of the gold salt coincided with that of the methyethylaminechloroaurate, but the chloroplatinate melted seventeen degrees higher than that of the methylethylamine, while the analysis of the both salts could scarcely show any difference in these two amines.

The decision could be given, if sinomenineiodomethylate was fused with kali. For, in this case, the amine to be liberated must naturally be tertiary and the ethanolamine, if it was produced at all, could not loose water intermolecularly.

In this supposition sinomenineiodomethylate was boiled with 66 per cent caustic kali in the same way as with sinomenine, and the sinomenol was obtained in 40 per cent yield of the theoretical. But the amine, isolated in this case was undoubtedly dimethylethylamine (m. p. of the chloroaurate 222° with decomposition, Au=47.87 per cent; calc. as $C_3H_{11}NAuCl_4H$, Au = 47.81 percent and m.p. of the chloroplatinate 239° with decomposition, Pt=35.38 per cent; calc. as $(C_3H_{11}N)_2PtCl_6H_2$ Pt=35.22 per cent.). From this fact, it may be inferred that the amine obtained in the kali fusion of sinominine must have been methylethylamine and not methylvinylamine. The fact that the m. p. of its chloroplatinate seventeen degrees higher than that given in the literature was perhaps one of examples often met with auri-

and platini-chlorides of the lower fatty amines

The decomposition of sinomenine in the fusion with kali can, therefore, be expressed in the following scheme.



If so, sinomenol must be dioxydimethoxyphenanthrene ($C_{16}H_{14}O_4$) itself, and not its dihydrid , as was supposed in the previous report. The comparison of the analytical data with the theoretical shows also clearly this fact The molecular formulae of sinomenol and its derivatives, given in the same report, must, accordingly, be corrected as follows.

		Found		Calculated		Calculated on the basis sinomenol $C_{16}H_{16}O_4$	
		C	H	C	H	C	H
Sinomenol	$C_{16}H_{14}O_4$	70 71	5 83	71 08	5 22	70 58	5 89
Diacetyl-sinomenol	$C_{20}H_{18}O_6$	1 67 45 2 67 88 3. 67 32	5 54 6 24 6 00	67 79	5 12	67 41	5 62
Dibenzoyl-sinomenol	$C_{30}H_{22}O_6$	1. 75 59 2 75 39	5 39 5 15	75 28	4 62	75 00	5 00
Dibenzoyl-sinomenol-chinone	$C_{30}H_{20}O_8$	69 42	4 07	70 86	3 96	–	–
Dimethyl-sinomenol,	$C_{18}H_{18}O_4$	A. 72 59 B. 72 01	6 51 6 39	72 45	6 08	72 00	6 71

Sinomenine was methylated by diazomethane in status nascendi. Methylsinomenine, thus obtained, melts at 169° and contains three methoxyl groups (26.51 per cent; calc. as three methoxyl groups in $C_{20}H_{25}NO_4$, 27.11 per cent). Its hydrochloride melts at 265° and is very sparingly soluble in water. Methylsinomenine gives neither diazoreaction nor ferric chloride and potassium ferricyanide reactions. It gives a semicarbazone (m. p. 149–151°, N=14.54 per cent; calc. as $C_{21}H_{26}O_5N_5$ N=13,69 per cent.) so that the existence of the carbonyl and hydroxyl groups independently each other, could be shown on sinemenine itself.

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ABSTRACTS FROM THE ORIGINAL PAPERS.

Über einen durch Hydrolyse des Adenylthiozuckers der Hefe gebildeten schwefelhaltigen Zucker.

Von

U. Suzuki and T. Mori.

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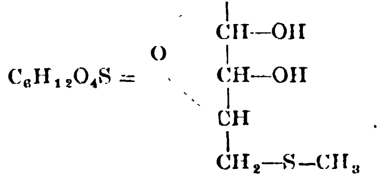
Einleitung.

Vor kurzem haben die Verfasser eine schwefelhaltige Substanz aus alkoholischem Extrakt der Hefe isoliert, welche durch Einwirkung von verdünnter Säure in Adenin und schwefelhaltigen Zucker gespalten wird. Das Phenylosazon des letztgenannten Zuckers krystallisiert in schönen gelben Prismen vom Schmelzpunkt 159–160°. Auf Grund verschiedener Beobachtungen wurde dem Thiozucker die folgende Formel vorgeschlagen. [U. Suzuki, S. Odake und T. Mori, Biochem. Zeitschr. Bd. 154, 278 (1924)].



$$C_6H_{12}O_4S = \begin{array}{l} CH{-}OH \\ | \\ CH{-}OH \\ | \\ CH{-}OH \\ | \\ CH \\ | \\ CH_2{-}S{-}CH_3 \end{array}$$

Die Verfasser haben die Untersuchung dieses Thiozuckers weiter fortgesetz und Folgendes gefunden.

1) Der freie Thiozucker ist ein hellbrauner Syrup und schmeckt schwach süsslich mit etwas bitterem Nachgeschmack; er gibt ausser allgemeinen Zuckerreaktionen noch die Pentosereaktion, nicht aber die Methylpentosereaktion, so gibt er z. B

beim Kochen mit starker Salzsäure dis Furfurolreaktion, gibt auch die Bial'sche sowie die Phloroglucin–Salzsäurereaktion, aber keine Blaufärbung mit rauchender Salzsäure und Vanillin. Er gibt ferner mit Aceton und rauchender Salzsäure eine Rotfärbung, welche aber sehr rasch verschwindet. (L. Rosenthaler, Der Nachweiss organischer Verbindungen II. Aufl. 194.)

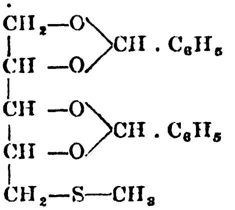
Diese Erscheinungen lassen sich dadurch erklären, dass die $-S-CH_3$ Gruppe zuerst durch Salzsäure abgespalten wird und der resultierende Zucker die eigentlichen Pentosenreaktsonen gibt.

2) Der Thiozucker absorbiert Brom, gibt weisse Fällung mit Quecksilberchlorid, Quecksilbernitrat und Goldchlorid.

3) Das Acetylderivat des Thiozuckers krystallisirt nicht, obgleich die Acetylzahl des möglichst gereinigten Präparats beinahe mit der des Triacetylderivats übereinstimmt.

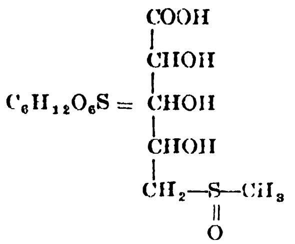
4) Das Dibenzoylderivat des Thiozuckers bildet farblose Nadeln von der Formel $C_6H_{10}O_4S\ (C_6H_5CO)_2$ mit dem Schmelzpunkt 185°.

5) Mit Natriumamalgam reduziert entsteht ein Thiozuckeralkohol von der Formel $C_6H_{14}SO_4$. Es bildet farblose büschelförmige Nadeln vom Schmelzpunkt 115–117°. Das Dibenzalderivat desselben bildet auch farblose Nadeln vom Schmelzpunkt 135–136°.



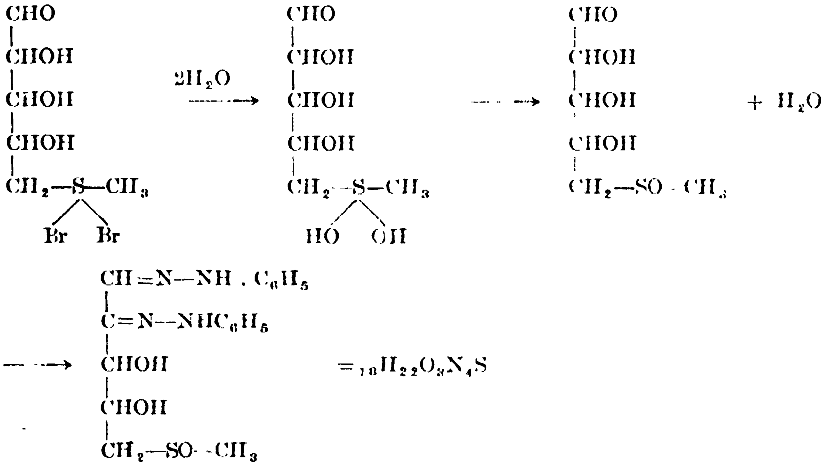
Dibenzalderivat des Thiozuckeralkohols.

6) Wird der Thiozucker mit konz. Salpetersäure unter Zusatz von Spuren Vanadinsalz vorsichtig eingedamft, so bilden sich etwa 32% Oxalsäure. Nimmt man aber die Oxydation mit verdünnter Salpetersäure (d=1, 15) ohne Zusatz von Vanadin vor, so entsteht sine Monocarbonsäure von der Formel $C_6H_{12}O_6S$ mit dem Zersetzungspunkt 183–184°. Sie ist keine Lactonsäure und absorbiert kein Brom. Mit Zink und Salzsäuse erhitzt, entsteht ein Mercaptanähnlicher Geruch. Höchst wahrscheinlich wird die $-S-CH_3$ Gruppe durch Salpetersäure zur $-SO-CH_3$ Gruppe oxydirt und die letztere wieder durch Zink und Salzsäure zum $-S-CH_3$ reduziert und weiter abgespalten. So nehmen die Verfasser für diese Monocarbonsäure die folgende Formel an:



$$C_6H_{12}O_6S = \begin{array}{l} COOH \\ | \\ CHOH \\ | \\ CHOH \\ | \\ CHOH \\ | \\ CH_2{-}\underset{\overset{\|}{O}}{S}{-}CH_3 \end{array}$$

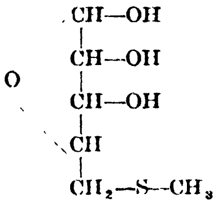
7) Wird die wässrige Lösung des Thiozuckers in der Kälte tropfenweise mit Brom versetzt, bis die gelbbraune Farbe nicht mehr verschwindet, dann vorsichtig mit Alkali neutralisirt und mit Phenylhydrazin erwärmt so entstehen schöne hellgelbe Prismen von der Formel $C_{18}H_{22}O_3N_4S$ mit dem Zersetzungspunkt 223-224°, welche Verbindung als Phenylosazon des sulfoxydirten Zuckers zu betrachten ist. Der Reaktionsverlauf ist wahrscheinlich folgender. (Hantzsch und Hibbert Ber. 40, 1514(1907).



Dass die Aldehydgruppe durch diese Behandlung nicht angegriffen, wurde durch Kontrollvrsuche mit Xylose nachgewiesen. Das Brom wurde nämlich in der Kälte von Xylose nicht absorbiert, sodass keine Oxydation stattgefunden haben kann.

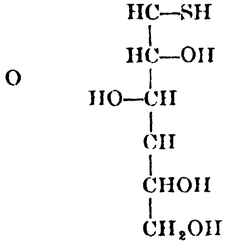
8) Die Bestimmung der S CH_3 Gruppe im Thiozucker resp. im Adenylthiozuckermolekül wurde nach Kirpal (Hans Meyer, Lehrbuch der Organisch-chemischen Methodik 4. Aufl. Bd. I 902-904 und 1098) und nach Kirpal und Bühn (Houben-Weyl, Die Methoden der Organischen Chemie II. Aufl. Bd. III 148-149) ausgeführt. Das Resultat war positiv, obgleich die Reaktion nicht quantitativ verlief.

9) Auf Grund oben erwähnter Beobachtungen kann man mit ziemlicher Sicherheit darauf schliessen, dass die Strukturformel des Thiozuckers:



ist. Das optische Verhalten soll später studiert werden.

10) Es sei noch bemerkt, dass Schneider und Wrede (Ber 47, 2225 (1914). Ber. 52, 1756 (1919); Zeitschr. f. Physiol. Chem. 119, 46 (1922); 126, 211 (1923) durch Spaltung des Sinigrins mit Kaliummethylat eine Thioglucose erhielten. Diese Thioglucose ist mit unserem Thiozucker nicht identisch, weil bei der Behandlung der ersteren mit Phenylhydrazin in der Hitze unter Glucosazonbildung leicht der Schwefel abgespalten wird.



Thioglucose Schneiders.

On the pupa oil of the tussah silkworm

(***Antheraea Peryni.***)

by J. Katō.

The pupa oil was extracted with ether from the pupa of the tussah silkworm in the author's laboratory, which was produced at South manchuria.

1. The physical and chemical properties were determined as follows:

Specific gravity at 20°C	0.9250
Melting point	12°C
Solidifying point	7°C
Refractive index. (by Abbe's refractometer.)	1.7464
Acid value	81.84
Saponification value	196.77
Ester value	114.93

Iodine value (by the Wijs' method.)	135.93
Reichert meissle value	1.38
Hehner value	93.32%
Unsaponificable matter	3.06%
Oxy acid	0.86%
Glycerol	9.82%
Acetyl value.	15.16

2. The mixed fatty acids were separated by saponification, and it was found that they contained no sterols. From these fatty acids the liquid and solid fatty acids were separated by the lead-salt ether method. Their properties were determined with following results.

	melting point	neutralization value	mean molecular weight	Iodine value
Mixed fatty acids.	32°	201.63	278.27	140.94
Solid fatty acids.	55°	219.72	255.34	0
Liquid fatty acids.	—	196.40	285.68	171.62

The mixed fatty acids contain 36.37 % of the solid fatty acids and 63.33 % of the liquid fatty acids.

3. The greater part of the solid fatty acids was the isopalmitic acid (m. p. 58-59°C), besides small quantity of the myristic acid (m.p. 53°C). was found.

4. The liquid fatty acids consisted of about 83% of the oleic, about 13% of the linolenic and small quantity of the linolic acid. By the bromination, 45 gm of the hexa bromostearic acid and 130.5 gm of the dibromostearic acid were isolated from 100 gms of the mixture. The hexa bromostearic acid was white powder, melting at 182°C. By the oxydation of the liquid fatty acids with the Hazura's method the dioxystearic, linusic, Isolinusic, azelaic and sativic acid were identified.

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JOURNAL OF THE AGRICULTURAL CHEMICAL SOCIETY OF JAPAN.

Vol. 1, No. 10 July, 1925. No. 10

ABSTRACTS FROM THE ORIGINAL PAPERS.

On the Differences between the Oryzenin of Common and Glutinous Rice.

T. Tadokoro, Y. Nakamura and S. Watanabe.

It is widely known that the difference between common and glutinous rice is caused by the different physico-chemical properties of the starches found in the two varieties while the quantitative differences of fat and protein contents have also been reported. Many authors investigated on rice proteins and distinguished four different kinds i. e. albumin, globulin, prolamin and glutenin which last is called oryzenin in the case of rice. Also many kinds of amino-acids in their decomposition products have been described. But until recently, there has been no report stating the difference between these two varieties of rice in respect to physico-chemical properties of oryzenin. Recently Kondo investigated only on the optical properties of rice protein which was extracted by alkalins and reported that the protein of common rice is more compact optically than that of glutinous.

The authors undertook the following experiments on many samples of both kinds of rice which were produced in different parts of Japan. (A) The distribution of these four kinds of protein, albumin, prolamin, globulin and oryzenin was determined quantitatively. (B) Physico-chemical investigations were undertaken on pure oryzenin which had been freed from albumin, globulin and prolamin. From these experiments, the authors found out many characteristic differences between common and glutinous rice oryzenin and here those results will be stated briefly.

(A) The chemical characteristics of oryzenin.

(1) On the distribution of four kinds of protein in both kinds of rice, the oryzenin contents predominate in glutinous rice.

(B) The physicochemical characteristic properties of oryzenin.

(1) The pure oryzenin contains from 0.291 to 0.599% of ash but in common rice oryzenin the ash content is always greater.

(2) The iso-electric point of glutinous rice oryzenin is more acidic than that of common, i. e. that of the former is in pH 4.8-5.2 and that of the latter is in pH 5.2-5.8.

(3) The solubility of glutinous rice oryzenin in alkali solution is greater than that of common rice oryzenin and the alkali solution of the latter is more turbid than that of the former.

(4) The viscosity of glutinous rice oryzenin in alkali solution is a little less than that of common rice oryzenin solution and the decrease of viscosity of the former by time is more pronounced than that of the latter.

(5) The rotatory power of glutinous rice oryzenin solution is lower than that of common rice. When this alkali solution was illuminated by ultraviolet rays its rotatory power decreases more rapidly in the case of the former.

(6) On the elemental composition of oryzenin, the nitrogen contonts of common rice is higher than that of glutinous rice. The difference is not only in the nitrogen contents but also in the sulphur and phosphorous contents which are reversed in the two kinds of oryzenins, i. e. the S-content of common rice oryzenin predominates and the P-content of glutinous rice oryzenin predominates.

Further difference was observed in their elemental composition. If the ratio of carbon for oxygen was taken, that of glutinous rice oryzenin is lower than that of common rice.

(7) In the partition of amino-acids in hydrolytic products of oryzenin, ammonia-, arginin- and lysin-form nitrogen are predominant in common rice oryzenin while monoamino-, histidin- and cystin-form nitrogen predominate in glutinous rice oryzenin.

(8) There is no remakable difference in tyrosin and tryptophan contents between the hydrolytic products of common and glutinous rice oryzenin.

(9) The iodine contents of oryzenin-iodide is superior in glutinous rice oryzenin in comparison with that of common.

(10) The free amino nitrogen content of common rice oryzenin is greater than that of glutinous and when the oryzenin alkali solution was illuminated by ultraviolet ray, the free amino nitrogen contents of glutinous rice oryzenin increased

more easily than that of common.

(11) In the pancreatin digestion of oryzenin, the glutinous rice oryzenin is digested more easily than that of common.

(12) The silver salt of glutinous rice oryzenin contains more silver than that of common but the nitrogen content is the reverse.

(13) The combined HCl–quantity of common rice oryzenin is greater than that of glutinous, because the former contains more amino groups in its molecule than the latter.

(14) The refractive index of common rice oryzenin is higher than that of glutinous.

(15) The contents of acetyl–group and of nitrogen in acetyl oryzenin are quite different between common and glutinous rice oryzenin, the former being superior in nitrogen contents and inferior in acetyl–group while the latter is the reverse.

(16) In the decomposition products of acetyl oryzenin, the common rice oryzenin produces great quantities of base, pyrrol, pyrrolic acid, $H_2OK_2CO_3$ soluble substances while the glutinous produces large quantities of pyrrolidin, glyoxalin and proteol.

Studies on Proteins. (The preliminary report)

(Contribution No. I from the Laboratory of Nutritional Chemistry, Department of Agricultuve, Kyoto Imp. University)

By Kinsuke KONDO.

A. In the writer's opinion, it is not right in the views of endeavouring to elucidate the essential or all nature and behavior of the colloidal solutions as those of proteins by the capillary chemistry, and also not by the physical chemistry nor pure chemistry only, rejecting the capillary chemical· consideration. Of course we can find many phenomena, which should be elucidated from the physico–chemical views, in the case of emulsoids such as protoin solutions, whose nature is near to the real solutions.

We must, therefore, deal with protein solutions, on the one hand, by stoichiometrical and physico–chemical measurements and particularly by the developement of more exact analytical procedures, and on the other hand, endeavour to elucidate the results of measurements under colloidal chemical as well as purely chemical consideration. This, the writer believes, is important on the progress of the protein chemistry.

B. The theory enounced by F. G. Donnan concerning the state of equilibrium in the case of semi-permeable membranes has been explained, for purposing to get a general conception of this theory.

C. It must be determined through the measurements of [H$^{\bullet}$], in what way [H$^{\bullet}$] varies, when the amount of NaOH per g-equivalent of casein nitrogen varies with constant amount of NaCl and constant volume. If we take the amount of NaOH (expressed in g-equivalent), which bound to g-equivalent of casein nitrogen, as ordinate, and [H$^{\bullet}$] (perhaps expressed in log-function) as abscissa, we can get some curves, each of these, corresponding to a certain NaCl-concentration.

When we get the full analytical results of our solutions, under using the above curves we can calculate in what way NaOH distributes between casein and dispersion medium. Applying these curves and the Donnan's theory to the results of measurements of osmotic pressure, the writer has shown that we can theoretically and experimentally find the molecular weight of casein.

(May 17, 1925)

A Method for the Preparation of Fumaric and Succinic Acids.

By

Teizo TAKAHASHI and Kinichiro SAKAGUCHI.

The ordinary method for the preparation of fumaric acid is to heat malic acid at 140–150°C for a long time. Recently Carl Wehmer patented (Brit. Patent 14641. Jonru. of chem. Soc. No. 710. Dec. 1921) a benefit way to prepare this acid from sugar by the fermentation of Asper. fumaricus (Asp. javanicus).

The authors, as reported already, have found ont the conditions optimum for the formation of fumaric acid by Rhizopus species, although C. Wehmer and F. Ehrlich have mentioned the formation of the acid by the other species of the same germs.

On the preparation of succinic acid suitable for the large produce few methods may be mentioned such as :– First, by the dry distillation of amber, secondly by the fermentation of Ca–malate with putrified casein (Liebig), thirdly by the fermentaion of am–tartarate (König), fourthly by the bacterial fermentation of citrate (U. Terada), fifthly by the oxydation of glutamic acid with nitric acid in presence of vanadinm (U. Suzuki, Y. Matsuyama).

Authors have prepared this acid most profitably by the reduction of fumaric acid, which is prepared from starch.

Experimental.

Fumaric acid preparation.

Fungus :– Rhizopus G. 34. Yamazaki.

The medium for the culture :– Water 1000g, Starch 100g, K_2HPO_4 0.015g, KH_2PO_4 0.015g, $MgSO_4$ 0.010g, $CaCl_2$ 0.010g, Urea 5g, Ca–carbonate 50g, Fe_2Cl_6 and NaCl trace.

In the medium after 23 days culture at 26–30°C, the yield was :–

Crude Ca–fumarate	43.7g.
Free fumaric acid	33.47g.
and Starch, decomposed	83.7g.

In other instance with the same fungus in the same medium replacing glucose (100g.) and peptone (3g.) respectively instead of both starch and urea after 29 days culture the yield was 31.5g. as crude Ca–fumarate, i. e. 20.2g. as free fumaric acid and there was no sugar remained in the culture medium.

So the yield of the acid from starch is 39%, calculated from used raw material.

The yield from glucose was low by this instance but it may be increased up to even more than 35%, if we minimanise the amount of peptone as we have reported already.

Succinic acid preparation.

For the preparation of the succinic acid from fumaric acid, an electrical reduction with lead pole was most suitable. Free acid is reduced completely after 30 minutes in 0.2% olution in water or 5% in alcohol, by passing the current of 6 volt and 1 amphere. Ca–fumarate may subjected in the same current in 1% solution in water to complete the reduction in 50 minutes.

The yield of succinic acid from starch may attain even over 35%.

[This paper is read before the meeting held at Tokyo Imp. Uni. Agric. Fact. Oct. 1924.]

JOURNAL OF THE AGRICULTURAL CHEMICAL SOCIETY OF JAPAN.

Vol. 1, No. 11. August, 1925. No. 11.

ABSTRACTS FROM THE ORIGINAL PAPERS.

Studies on Proteins. I.

(Contribution No. 2. from the Laboratory of Nutritional Chemistry, Department of Agriculture, Kyoto Imp. University)

By Kinsuke KONDO.

A. A brief account is given of some of the most important researches hitherto published on the occurence of combination between protein and mineral acid or base. (1) One view says that the binding of protein to mineral acid (strong acid as HCl) or alkali is not chemical combining in a true sense, but only absorption phenomenon. (2) The other view says that protein and acid or alkali really combine chemically. In the latter view, we can find two branches. (a) The compounds of protein with acid or alkali do not yield on electric dissociation the simple anion of acid or cation of base, in one or more of which the inorganic radical is bounded up in a non-dissociable form. T. B. Robertson holds this opinion. (b) The compounds of protein with acid or alkali can yield on dissociation the simple anion of acid or cation of base in question. W. Pauli, S. P. L. Sörensen, J. Loeb hold this view. Examining the experiments, the different views grounded on, the latter opinion is most probable,……the writer believes……regarded from the point of modern view of the matter.

B. The casein employed in my investigations was explained. It was prepared by Kahlbaum according to the Hammarsten method. The water content, determined partly by drying in vacuo for 24 hours at 80°C, partly by standing for three months in a sulphuric acid desiccator, was 9.43%. The ash content and nitrogen content of the dried casein were 0.15% and 15.46% respectively. The method of nitrogen determination was explained briefly. The factor by which the quantity of nitrogen in the casein must be multiplied to give the corresponding weight of dried casein is thus 100/15.46 = 6.47.

C. The measurements of the solubility of casein in HCl revealed the peculiar fact that the solubility decreases……with the same concentration of HCl to begin with……as the quantity of precipitates in contact with the solution increased.

I have postponed the explanation of this feature until more comprehensive investigations have been made, but have just suggested that it should perhaps be sought in the capacity of casein to cause complex binding of chlorine ions both in the solution and together with hydrogen ions in the precipitate and solution being well calculated to explain the experimental results.

D. It was examined whether the casein in HCl–solution studied alters or not, through the determining of formol–titrable nitrogen. The results came to that the casein can keep the same nature in the solution of different concentration of HCl employed. It was found that the casein forming the casein chloride in HCl–solution occupies 0.891c.c. per 1g of dried casein.

E. In this section the writer has thrown one glance on the activity theory of ion.

(a) The theoretical considerations and experimental works were made for the determination of chlorine ion activity. The results came to the expression

$$pa_{Cl} = \frac{0.0674 - E_{Cl}}{0{,}0577} \cdots\cdots\cdots \text{at } 18°\text{C.} \cdots\cdots (1)$$

where $pa_{Cl} = -\log a_{Cl}$, and a_{Cl} is the chlorine ion activity in the solution and E_{Cl} is the potential of the following elements:

Hg, HgCl, 0.1n KCl | 3,5n KCl | Exp. liquid, HgCl, Hg. ……………………(I)

The measurements were carried out in a thermostat at 18°C.

(b) For determination of the activity of the hydrogen ion I got the following formula through precisely the same theoretical considerations and experimental results as with the determination of chlorine ion activity:

$$pa_{H} = \frac{E_{H} - 0.3348}{0.0577 + 0.0002(t - 18°)} \cdots\cdots\cdots (2)$$

where $pa_{H} = -\log a_{H}$, and a_{H} is the hydrogen ion activity in the solution and E_{H} the potential of the following element:

Hg, HgCl, 0.1n KCl | 3.5n KCl | Exp. liquid, H_2, Pt. ……………………(II)

The measurements were made with the Hasselbach's shaking electrode in an air thermostat.

(c) By measurement of hydrogen ion activity and chlorine ion activity in the solutions of casein-hydrochloric acid, and reckoning back to concentrations of these two ions after the activity theory, it was found that the casein in the solution binds per g (not dried) about 2.10 gram equivalents of chlorine ions, and dissociation degree of casein chloride is about 0.72. It will be also noted that the dissociation degree of casein chloride and the number of complex-bound gram-equivalents of chlorine ions per g casein, appear to be independent of the hydrogen ion activities used. (June 18, 1925.)

On Sulphur-containing Amino-acids. V.

On the Oxidation of Cystine and Cysteine by Iodine.

by YUZURU OKUDA and MUNEKUMA SAMEJIMA.

From the biochemical Laboratory, Department of Agriculture, Kyūshū
Imperial University, Fukuoka, Japan.

Friedmann (Beitr. Chem. Physiol. Pathol., 3, 1. 1902) has found that cystine as well as cysteine are oxidized by bromine into cysteic acid, and Okuda (J. Tokyo Chem. Soc., 37, 181, 1916, and J. Chem. Soc. Japan, 45, 1, 1924.) studied this oxidation quantitatively, and ascertained that in these cases one molecule of cystine requires 10 atoms of bromine and one molecule of cysteine 6 atoms, and applied these facts for the determination of them.

Lately, Okuda (J. Chem. Soc. Japan, 45, 1, 1924 and 19, 1924.) also found that among amino acids cysteine alone reacts with iodine very actively in an acid solution and at room temperature, and applied this fact for the determination of cystine and cysteine. But on the oxidation products in this case no further investigation was performed.

In the present investigation the following facts were ascertained :–

1. At higher temperature both cystine and cysteine are oxidized into cysteic acid, one molecule of cystine requiring 10 atoms of iodine and one molecule of cysteine 6 atoms of iodine for oxidation. **2.** At room temperature and in hydrochloric acid solutine, cystine does not react with iodine. But cysteine does, and the chief oxidation product is cystine, accompanyed by a little cysteic acid. The ratio of the quantities of the oxydation products depends upon the acidity and temperature of the sample.

*On the Application of Aging yeast (**Willia anomala**) to Saké and Saké artificial.*

By Teizo TAKAHASHI.

(Faculty of Agriculture, Tokyo Imperial University.)

It was about fifteen years ago that the writer has reported on the nature of aging yeast of saké and its application to the saké manufacture. (Journ. of the Callege of Agric. Vol. I. No. 3. p. 227-268, 1911). At the time it was applied, in pure culture, in the begining of "moto"-mash or at the stage of "Moromi"-mash or at the aging stage of saké, i. e. after the pressing of "Moromi"-mash.

The aging effect was most conspicuous when it was discovered. However, recently it happened that aging nature of this yeast to saké has almost been lost after too long series of successive generations in artificial cultare medium.

In 1921, the writer changed the medium to an incomplete one, devoid of carbohydrate which was replaced by ethylalcohol as shown below :-

Alcohol abs.	5 c.c.
Asparagine.	2.5 g.
$K\ H_2P_2O_4$.	1.0 g.
$MgSO_4$.	0.34 g.
Water.	95.0 c.c.

The culture in such a defective medium showed very good growth and gave favourable change for the ripening of young or unmatured saké. The change of chemical composition of saké durig aging by this yeast is shown below :-

	Saké, original.	Saké, aged.
P_H value.*	4.3	4.3
grams in 100 c.c.		
Extractive matters.	4.218	4.196
Total acids. as succinic acid.	0.215	0.223
Total nitrogen.	0.185	0.182
Protein matter. (Stutzer's method).	0.065	0.067
Dextrin	0.745	0.457
Reducing sugars.	1.48	1.248
Ethyl alcohol	17.24 vol %	17.64 vol %
Esters (as di-ethiyl succinate).	0.089	0.092

Colloid matters. Drum number.	1239	1206
Colloid number. •	66	45

* After L. Mihaelis' colorimetric method. (L. Mihaelis und. A. Gyemant. Bioch. Zeit. 109, 165. 1920.; L. Mihaelis und. A. Krüger. Bioch. Zeit. 119, 307. 1921.)

• Author's method. This journal vol. I. No. I.

The yeast do favours similary for the aging of artificial ones.

On the Refractometric Quantitative Analysis of Alcohol and the Extract in Sake.

By Mitsuji ITO.

Experiments on the refractometric quantitative determination of ethyl alcohol in spirituous liquor have been made by several authors, but so far its application for the analysis of *Sake*, a Japanese alcoholic beverage made of rice, has not been done.

In order to determine alcohol and the extract in *Sake* quantitatively by the use of Pulrich's refractometer, I have examined many kinds of *Sake* produced in various districts of Japan; and have found the following formulae for the calculation of alcohol and the extract indirectly. These resulting figures were compared with those obtained directly by the ordinary method.

I. For the calculation of alcohol in volume %.

$$X = \frac{R-(W+U\times E)}{D} \quad \cdots\cdots\cdots\cdots \text{Formula a.}$$

$$A' = X \pm x \cdots\cdots\cdots\cdots (1) \qquad U = \frac{r-r'}{e} \cdots\cdots\cdots\cdots (2)$$

Where, X = Calculated % of alcohol in volume.

A' = Required % of alcohol in volume.

x = Average supplemental number found from the examination of many kinds of *Sake*.

R = Refractive index of *Sake*, measured by the Pulfrich's refractometer at 20°C.

W = 1.33274, refractive index of water at 20°C.

E = Weight of extract, gram in 100 c.c. of *Sake*.

D = 0.0005, mean value of the refractive index corresponding to one volume % of alcohol at 20°C.

r = Mean value of the refractive index of *Sake*.

r′ = Refractive index of the alcohol solution, the concentration of which is equal to the mean value of that in *Sake*.

e = Mean value of the extrrct in 100c.c. of *Sake*, in gram.

U is calculated as follows:– 0.00192 is the mean value for 72 kinds of new and old *Sake*, 0.00190 for 59 kinds of old *Sake*. 0.00188 for 35 kinds of Honshū old *Sake*, 0.00189 for 31 kinds of Kwansai old *Sake*, 0.00193 for 24 kinds of Hokkaido old *Sake*, and 0.00203 for 13 kinds of Hokkaido new *Sake*. Using 0.00197 (mean value between 0.00190 and 0.00203) for the value of U, the above formula may be shown as follows.

$$X = \frac{R-(1.33274+0.00197\times E)}{0.0005} = \{R-(1.33274+0.00197\times E)\}\times 2000 \cdots \cdots \text{Formula a}'.$$

II. For the calculation of the extract, gram in 100c.c.

$$X = \frac{R-(W+D\times A)}{U} \cdots\cdots \text{Formula b.}$$

$$E' = Y \pm y \cdots\cdots (3)$$

Where, E′ = Requred weight of the extract in 100c.c. of *Sake*.

y = Average supplemental number found from the examination of many kinds of *Sake*.

A = Volumetric % of alcohol in *Sake* measured by specific gravity of the distillate as usual.

R, W, D and U are the same as the above.

The above formula b may be shown as follows.

$$Y = \frac{R-(1.33274+0.0005\times A)}{0.00197} = \{R-(1.33274+0.0005\times A)\}\times 508 \cdots\cdots \text{Formula b}'.$$

The values of X calculated for the many kinds of *Sake* by the formula above descrived are nearly consistent with those of A obtained by ordinary method, and the mean value of x between X and A is –0.24 % for Kwansai old *Sake*. 0.07 for Kwanto and Tohoku old *Sake*, 0.18 for Hokkaido old *Sake*, 0.61 for Hokkaido new *Sake*, and 0.06 for all these kinds of *Sake* mentioned above. A′ is calculated from these X and x above obtained.

In like manner values of Y are nearly consistent with those of E obtained by ordinary gravimetric way, and the mean value of founded between Y and E is –0.0546g. for Kwansai old *Sake*, –0.0013 for Kwanto and Tohoku old *Sake*, -0.0108 for Hokkaido old *Sake*, 0.1495 for Hokkaido new *Sake*; and –0.0002 for all these kinds of *Sake* above said. E′ is calculated from these Y and y above obtained.

From the above results, it may be stated that the practical use for the refractometric quantitative analysis of alcohol and the extract in *Sake* or other its analogous alcoholic beverage is to be applied. The difference between the values of alcohol obtained by the refractometric method and those by the ordinary method is rather smaller than the experimental error which occurs between the primary distillate and the redistillate.

Aldehyde in Connection with the Saké-brewing.

By Masakazu YAMADA.

Recent works of Neuberg, Neubauer, Kurono, Embden, Oppenheimer etc., have showed that aldehydes and ketones play an important rôle as the intermediate products in various phases of fermentation.

In the main fermentation of Saké-brewing aldehydes have also been observed everytime but little study was made on them.

The author studied them from the standpoint that they might have relation to the delicate flavour of saké and as well as to its keeping qualities.

The only aldehyde, obtained by steam distillation of saké was acetaldelyde. It was identified from the melting point of its *aldomedon*? of its paro-nitro-phenylhydrazone and also from the content of nitrogen of the latter. The content of acetaldehyde was estimated by Ripper's bisulphite method at each period of brewing and storage.

At the stage of "moto"—cultivation of Saké-Yeast—and "moromi"—mainfermentation of saké—the content of acetaldehyde was quite small and showed little variation. Fresh and old saké are easily distinguished by the estimation of the aldehyde content, for aldehyde increases gradually during the storage period, thus the average content being 0.0057 percent in the fresh saké in April and 0.01117 percent in the old in October.

From the antiseptic nature of the acetaldehyde, though not very strong it might be supposed that its quantity is in relation of the resistance of saké against "hiochi"-a putrefactive phenomenon of saké caused by some lactic and acetic acid producing bacteria. The content of the acetaldehyde has heen, in practice, measured by simply warming a portion of saké at intervals of about a month during the storage period. This is called "nomikiri" In the putrefied saké only trace of acetaldehyde is to be met with. It is imaginable that the acetaldelyde will be oxidised into acetic acid in putrefaction process, because the acid content of putrefied saké is ordinarily higher than that of the healthy one. Endly, aldehyde has nothing to do with the quality of saké.

In "shōchū"—Japaese whisky prepared by destilling the saké-cake—the flavaur is accurately proportional to its aldehyde content.

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ABSTRACTS FROM THE ORIGINAL PAPERS.

On the Products of Partial Hydrolysis of the Silkfibroin.

By Hisashi ARIYAMA.

(Received June 14th, 1925)

In 1923 W. S. Ssadikow and N. D. Zelinsky (Biochem. Zeitschr., 136. 241; 143, 504; 147. 30) reported on a new method for hydrolysing the protein. Their method consists in the following two principal processes: (1) hydrolysis of the proteins by heating them with 1 % HCl in autoclave at high temperature for several hours (2) successive extraction of resulting products with organic solvents. By this method they obtained from goose feather and horse hair various kinds of anhydride of amino acids. The writer applied this method to the silkfibroin, slightly modifying the conditions, and isolated as the decomposition products four kinds of anhydride, in which glycine, alanine and tyrosine were the constituents respectively.

Hydrolysis of the silkfibroin :–

1 kg silkfibroin was heated with 8 times its weight of 0.5% HCl in autoclave for 8.5 hours at 170°C. After filtered from the volminous undissolved matter (300g. in dry state), the solution was neutralized with dilute NaOH and was concentrated to one-tenth of the original volume below 40°C.

Isolation of the anhydrides:–

Crystal A (2 tyrosine + 2 glycine + 1 alanine anhydride):

This separated as crystalline plates during the operation of concentrating the neutralized solution. Decolorized and recrystallized several times from hot water, it crystallized in heavy transparlent plates, m. p. 255–258°C (incorr). It is almost insoluble in ordinary organic solvents, but is soluble in water and glacial acetic acid when warmed and gives Millon's-and picric acid reactions (anhydride reaction), but biuret-and ninhydrin reactions are absent.

Complete hydrolysis of this compound gave tyrosine and the mixture of glycine and alanine. From these results, as well as from the results of elementary analysis, Crystal A may be regarded as an anhydride consisted of 2 mol. tyrosine + 2 mol. glycine + 1 mol. alanine.

Crystal B (glycyl-l-tyrosine anhydride):

The concentrated solution, after being filtered from Crystal A, was extracted with acetic ether in Dakin's extration apparatus for 35 hours. From this extract Crystal B was obtained as fine white needles, m. p. 267–275°C (incorr.), and $[\alpha]_D^{20} = +100.2^\circ$ in ammoniacal solution. It is insoluble in ether and $CHCl_3$, soluble in warm alcohol and water and easily soluble in dilute NH_4OH and glacial acetic acid in the cold. As regards its color reactions, Crystal B behaves just analogously to Crystal A.

On evaporating the mother liquor of the recrystallization of crystal B, a further crop of white needles was obtained which may be called here Crystal C. From the results of elementary analysis and various properties, Crystal C may be regarded as almost identical with Crystal B, except that it shows a little difference in its melting point, ie. 282–285°C (incorr).

Crystal D (glycyl-alanine anhydride):

When the filtrate of Crystal B was evaporated down in vacuo, yellowish solid substance separated out which was taken in warm acetic ether. On cooling the acetic ether solution, Crystal D separated out as transparent heavy crystals, m. p. 242–243°C (incorr) and $[\alpha]_D^{20} = +5.7^\circ$ in aqueous solution. It is soluble in water and glacial acetic acid, but rather difficultly in alcohol, methylalcohol and ether. It gives strong picric acid reaction while ninhydrin-and Millon's reactions, are absent. Cryoscopic measurement in Water gave the molecular weight of 131 (theory 128).

By the hydrolysis, Crystal D gave glycine and alanine.

Crystal E (glycyl-d-alanine anhydride): This was obtained from the filtrate of Crystal D and recrystallized from warm methyl alcohol in very fine white needles, m. p. 245–247°C (incorr.) and $[\alpha]_D^{20} = -3.8$ in aqueous solution. In the solubility and color reactions Crystal E is almost analogous to Crystal D and also the results of elementary analysis stand close to that of Crystal D. The complete hydrolysis of Crystal E gave glycine and alanine. Measusement of its molecular weight gave close value to that af Crystal D, i. e. M=134 (Crystal D. 131).

While on the various points, as above mentioned, these two crystals are almost analogous, they differs as to the crystall form and rotatory power each other, and these differences may be due to the degree of racemization of these compounds during operations.

Of these four kinds of anhydride, Crystal B (C) (glycyl-l-tyrosine anhydride) and Crystal E (glycyl-d-alanine anhydride) are identical with those previouly obtained from the silkfibroin by F. Fischer and E. Abderhalden while as to the isolation of the other two anhydrides, Crystal A (2 tyrosine + 2 glycine + 1 alanine anhydride) and Crystal D (dextro rotatory glycyl-alanine anhydride) it seems that nothing has been reported up to present.

Studies on Proteins. (II).

By Kinsuke KONDO.

(Contribution No. 3. from the Laboratory of Nutritional Chemistry, Department of Agriculture, Kyoto Imp. University) (Received July 1st, 1925)

A. It has been shown by surveying the beautifull works of S. P. L. Sörensen and J. Loeb that the Donnan theory has been of great importance in the developement of the theoretical chemistry of proteins. Viewed in the light of the activity theory, the facts are in reality very little altered. The electric potential difference E_M is mearly determined here by

$$E_M = \frac{RT}{F} \ln \frac{a'}{a''} \quad \cdots\cdots (1)$$

where a' and a'' represent the activity in a state of equilibrium of one of the monovalent diffusible ions on the one side and on the other side of the membrane. And if we take sodium chloride as the only diffusible electrolyte, Donnan's simple equation

$$C'_{Na} \cdot C'_{Cl} = C''_{Na} \cdot C''_{Cl}$$

is altered accordingly to

$$a'_{Na} \cdot a'_{Cl} = a''_{Na} \cdot a''_{Cl} \quad \cdots\cdots (2)$$

B. The methods and apparatus serving for determining the membrane potential of casein chloride solution are described in detail. The measurement of the membrane potential is effected by means of the following element:

Hg, HgCl, 0.1n KCl | 3.5n KCl | Prot. sol. || Membrane || outer liquid | 3.5n KCl | 0.1n KCl, HgCl, Hg.

where the protein solution contains, in addition to the protein, also the diffusible ions with activities associated with the corresponding activities in the outer liquid by means of equations such as (1) and (2).

C. In this sectionare set forth several experiments intended to show how rational and constant the osmotic pressures of casein chloride and sodium caseinate solution are measured by means of the method worked out chiefly by J. A. Christiansen (see Studies on Proteins, S. P. L. Sörensen, Comptes-Rendus du Laboratorie Carlsberg, 12) Here also the activity of hydogen ions was measured in the inner and outer liquid a after the experiment.

D. The experimental results shew that both osmotic pressure and membrane potential of the casein chloride solution decrease with increasing activity of hydrogen ions. The cause, or at any rate the main reason, of the ascent of the osmotic pressure and membrane potential is sought in the two fold action of the hydrochloric acid, an ionising effect proceeding from the hydrogen ion, and a salting out effect proceeding from the chlorine ion, which has altogether a specific effect on casein.

(June 25, 1925)

Photoaktivierung von Vitamin A, Cholesterol, von Fetten und anderen Substanzen durch Ultraviolettstrahlen.

Von

S. HAMANO.

(Aus dem biochemischen Laboratorium des Instituts fur physikalische und chemische Forschung zu Tokio.) (Received July 1st, 1925)

Vor zwei Jahren hat K. Takahashi(1) zum ersten Male beobachtet, dass das von ihm dargestellte, "Biosterin" (gereinigtes Vitamin-A Präparat) in vollkommener Dunkelheit auf photographische Platten wirkt, Gleich darauf hat der Verfasser gemainsam mit ihm gefunden, dass diese Lichtwirkung durch Bestrahlung mit Ultraviolottstrahlen bedeutend verstärkt wird. Ferner wurde festgestellt, dass Cholesterol, Olivenoel u. s. w., welche ursprünglich nur schwache oder kaum merkbare Wirkung

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(1) Journ. Chem. Soc. Japan, Vol. 44, 59; Journ. of the Institute of Physical and Chem. Research Vol. III, No. 6 (Dec. 1924), 693-761.

haben, durch Bestrahlung stark aktiviert werden, während andere Substanzen wie Stärke, Zucker, Aminosäuren u. s. w. nicht solche Eigenschaften zeigen.

Dieser merkwürdige Befund wurde Ende Dezember 1924 von Takahashi in der Japanischen Agrikultur-chemischen Gesellschaft vorgetragen. Leider ist er kurz nachher gestorben, sodass der Verfasser das Studium weiter fortgesetzt hat, um diese Versuche zu erweitern und zu vervollständigen.

Es ist eine wohlbekannte Tatsache, dass die Ultrastrahlen auf die Ernährung der Tiere, besonders auf die Heilung von Rachitis grossen Einfluss hat. In neuester Zeit ist sogar eine interessante Beobachtung von Steenbock, Hess und Rosenheim u. A.(2) mitgeteilt, dass rachitische Ratten nicht unr durch direkte Ultrastrahlen, sondern auch durch blosses Zusammenbringen mit den ultrabestrahlten Ratten in einem Käfig von der Krankheit geheilt werden. Ferner wurde berichtet, dass Cholesterol durch Bestrahlung die antirachitische Wirkung entfaltet. Das künstliche Futtergemisch soll nach den oben genannten Autoren durch Bestrahlung gegen Rachitis wirksam werden.

Wie kann man diese Erscheinung erklären, wenn dies tatsächlich der Fall ist?

Takahashi hat schon darauf aufmerksam gemacht, dass die eigentliche Wirkung des "Biosterins" auf Tiere mit der Photoaktivität beinahe parallel geht. Geht die Vitaminwirkung des Biosterins verloren, so hört die Photoaktivität ebenso auf. Er nahm deshalb an, dass entweder irgend eine unbeständige auf die photographische Platte hochwirksame Substanz (Gase?) stets aus Biosterin ausgestrahlt wird, oder die labile Atomgruppe in Biosterin die umgebende Luft aktiviert. Cholesterol, Fette und andere Substanzen sollen durch Bestrahlung eine unbeständige Atomgruppe in ihrem Molekül bilden, welche direkt oder indirekt auf die photographische Platten wirken und gleichzeitig die antirachitische Eigenschaften entfalten. Wenigstens steht die antirachtische Wirkung und Photoaktivität in innigem Zusammenhang. Der Verfasser wird aber gegenwärtig auf solche Hypothese nicht eingehen, sondern nur die experimentallen Tatsachen mitteilen.

Experimentaller Teil.

Als Quelle der Ultraviolettstrahlen wurde, auf freundlichen Vorschlag von Prof. H. Nagaoka, dessen Quecksilberlampe (Ordinary pressure mercury lamp), die kürzlich von ihm in diesem Institut konstruiert wurde, angewandt. Diese Lampe (Fig. 8) besteht aus einem U-förmig gebogenen durchsichtigen Quarz rohr, welches an seinen beiden Enden wieder aufwärts gebogen ist. Das Leuchtrohr stellt einen schmalen Kanal dar. Die kugelförmigen Reservoirs an beiden Enden sind mit dem Leuchtrohr durch feine Kapillare von etwa 40 cm. Länge verbunden. Man füllt das Rohr mit Quecksilber, leitet einen elektrischen Strom von 1 Ampere und 500 Volt und erhitzt vorsichtig des Leuchtrohr mittels eines Breuners. Es entsteht dadurch Quecksilberdampf im Leuchtrohr, die Entladung findet statt, und es entstehen die ultravioletten Strahlen. Die Länge des Lichtbogens ist 4 cm. Man kann diese Lampe 20–40 Stunden lang kontinuierlich in Betrieb erhalten.

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(2) Steenbock and Daniels (1925) J. Amer. Med. Ass. No. 15 Vol. 84, 1093.
Hess and Weinstock (1925) Jour. Biol. Chem. 63, 305.
Vergl. Literaturangaben am Schluss der Arbeit.

Versuche mit Cholesterol.

Zu diesem Zweck wurden 2g sorgfältig gereinigtes und getrocknetes Cholesterol in ein Quarzrohr von 15cm Länge und 1.5cm. Durchmesser gebracht, mit Kork verstopft, mit Dehkotinsky'schem Cement abgedichtet und in einer Entfernung von 20cm von der Lichtguelle 24 Stunden ununterbrochen bestrahlt. Man bringt darauf 1g. desselben in eine Glasschale (d=3cm; h=1.6cm) und bedeckt sie mit einer pohtographischen Platte (Lion Express, Ortho) Die Entfernung der Platet von der Substanz betrug etwa 1cm. Man belässt die Glasschale in absolut dunklem Raum 24 Stunden. Dann wurde die Platte herausgenommen und das Negativ in gewöhnlicher Weise entwickelt. Zur Kontrolle wurde das Cholesterol ohne vorherige Bestrahlung genau so behandelt. Der Unterschied zwischen beiden Bildern ist aus Fig. 3-5 ersichtlich.

Auf diese Weise wurden noch verschiedene andere Substanzen geprüft. Das Resultat war folgendes:

1. Cholesterol, Lebertran, Olivenöl, Ölsäure und deren Calciumsalz, Zimmtsäure, Abietinsäure, Kampher, Borneol, Menthol, Balsam, etc. verhielten sich genau so wie Cholesterol wie man aus Fig. 1–4 sieht.
2. Wurde das Quarzrohr in oben erwähnten Versuchen entweder evacuiert oder mit Wasserstoff gefüllt, so war die Wirkung auf die photographische Platte viel schwächer als in den Experimenten, in denen das Quarzrohr mit Luft oder mit Sauerstoff gefüllt war. (Fig. 5). Die Feuchtigkeit hat in diesem Versuche keinen merkbaren Einfluss gezeigt.
3. Lässt man das photoaktivierte Cholesterol in dunklem Raum stehen, so geht die Aktivität allmählich verloren. So war es noch nach 26 Stunden aktiv,

aber nach 98 Stunden war es völlig inaktiv. (Fig. 6).

4. Wenn man gewöhnliches Glasrohr an Stelle des Quarzrohrs gebraucht, so ist die Aktivität viel schwächer, was vielleicht durch Absorption der ultravioletten Strahlen durch Glas verursacht wird. (Fig. 7).
5. Traubenzucker, Rohrzucker, Stärke, gesättigte Fettsäuren Bernsteinsäure, Korksäre, Aminosäuren, Salicylsäure, Anthracen, Naphthalin, Cetylalkohol, etc. hatten selbst nach Bestrahlung keine Photowirkung.

Literatur:

1. Steenbock and Daniels (1925) Jour. Amer. Med. Assoc. No. 15, Vol. 84, 1093.
2. Hess and Weinstock (1925) Jour. Biol. Chem. 63, 305.
3. Rosenheim and Webster (1925) Lancet No. 20, Vol. L, 1025.
4. Steenbock and Black (1924) Jour. Biol. Chem. 61, 405.
5. Nelson and Steenbock (1955) Jour. Biol. Chem. 62, 575.

An dieser Stelle spreche ich Herrn Prof. Dr. H. Nagaoka, Dr. R. Asada, Dr. T. Machida und Herrn Prof. Dr. U. Suzuki meinen aufrichtigen Dank für die freundlichen Ratschläge aus.

On Vitamine C in Satsuma Orange (Oonshiu Mikan).

Y. IWASAKI.

(Received July 1st, 1925)

The author found that vitamine C content in the juice of the flesh of Satsuma orange was conciderably rich. The method used for the experimental scurvy was applied to the same one which the author described in previous paper (Y. Iwasaki: J. Agr. Chem. Soc. Japan. I; 80, 1925).

When 7c.c. of the juice were added every day to the fodder of the animals, this quantity could completely cure the animal scurvy in 50 days, but 5c.c. of the juice could not cure entirely, and the animals had still very slight symptoms of scurvy at 50 days after the juice had been added. In the case of 3c.c., the animals had more signs of the disease than those to which 5c.c. were given. And one animal died after 30 days. The animals which had been given 1.5c.c. every day could not keep their lives and all died before 50 days. The autopsies showed many scurvy symptoms.

So it can be said that daily minimum doze of 4-5c.c. of the juice of the Satsuma orange is sufficent to cure scurvy of guinea-pig.

Pri la kristalformado de mielo.

de

S. Sawamura kaj R. Sasaki.

(Received June 10th, 1925)

Kristalformiĝo de mielo estas akcelata nek de la ĉeesto de aero, nek simple de malalta temperaturo, sed ĝi facile okazas en manko de aero, se ni aldonas kelke da ĝiaj kristalojn al mielo, kaj kontraŭe neniom da kristaloj estas aldonata, mielo restas tute diafana dum longa tempo, eĉ en la ĉeesto de aero. La kaŭzo de kristalformiĝo estas sekvanta:

Mielo, sin algluanta sur la muro de ujo, elsekiĝas, kaj tiel tie ekformiĝas kelke da kristaloj. Kiam jam ekformiĝas iom kristalaĵoj, tiam ili kondukas la tuton al la kristaliĝo, ĉar mielo estas supersaturigita per sukero. Tial por malhelpi kristalformiĝon, unue mielo devas esti varmigata al 40°C, por ke la kristaloj jam ekzistantaj, sin dissolvu, kaj due oni devas tute plene enmeti la mielon en la ujon, por ke ĝi ne havu la okazon kristalformiĝi per sekiĝado sur la muro de la ujo, kaj laste ĝi devas tre bone enŝtopata.

Über das Vorkommen von Methylmercaptan in frischer Raphanus Wurzel (Daikon, Raphanus Sativus L.)

Von

Nobuzo Nakamura.

(Aus dem Agrikulturchemischen Institut der Universität Tokio.)

(Received July 2nd, 1925)

Dass durch Fäulnis des Eiweisskörpers Methylmercaptan gebildet wird, ist eine altbekannte Tatsache. Das Vorkommen desselben in frischem Pflanzengewebe ist

aber bis jetzt von niemandem bewiesen. Wenn man frische Raphanuswurzel fein zerreibt und, wie es bei uns Sitte ist, den Brei derselben (Daikonoroshi) bereitet, so entwickelt sich ein eigentümlicher Geruch.

Nänert man Bleiacetatpapier, so bildet sich darauf in kurzer Zeit ein gelblich brauner metallglänzender Film.

Wird der Brei in einem Rundkolben vorsichtig erwärmt und unter langsamer Luftleitung bei vermindertem Druck distilliert, und der entwickelte Dampf in Aethylalkohol eingeleitet, so wird die flüchtige Substanz vom Alkohol aufgenommen. Diese Lösung riecht stark nach dem ursprunglichen Brei und gibt folgende Reaktionen:

1.) Mit alkalischer Nitroprussidnatriumlösung gibt sie Violettfärbung, die durch Zusatz von Säuren verschwindet und durch Alkali wieder zum Vorschein kommt.

2.) Mit wässeriger Quecksilbercyanidlösung gibt sie weisse Fällung.

3.) Mit Bleiacetat entsteht gelbe Fällung.

4.) Mit Quecksilberchlorid entsteht eine weisse Fällung.

Diese Reaktionen lassen das Vorhandensein von Methylmercaptan leicht erkennen. Um es als charakteristische Quecksilberverbindung zu isolieren, hat der Verfasser mit grösserer Breimenge gearbeitet. Zu diesem Zweck wurde der Brei in einem grossen Rundkolben mit Oxalsäure angesäuert und das Ganze unter langsamer Luftleitung bei vermindertem Druck der Distillation unterworfen. Das Distillat wurde in eine dreiprocentige wässerige Quecksilbercyanidlösung eingeleitet. (Der Zusatz von Oxalsäure hat den Zweck, die Austreibung des Mercaptans zu beschleunigen. Siehe; Fränkel, Praktikum der medizinischen Chemie).

Der dabei entstandene weisslich gelbe Niederschlag wurde abgesaugt und im Exsiccator über Chlorcalcium getrocknet. Wird diese Substanz mit viel Aceton heiss extrahiert, so geht ein Teil in Lösung und nach dem Erkalten scheiden sich perlmutterglänzende weisse Krystalle von Quecksilbermercaptan aus. Der in Aceton unlösliche gelbe Rückstand löst sich in den gewöhnlichen Lösungsmitteln wie Alkohol, Methylalkohol, Ligroin, Aether, Eisessig, Benzol und Toluol nicht, sodass der Verfasser kein geeignettes Mittel finden konnte, um ihn zu reinigen. Es wurde nur bestätigt, dass darin Kohlenstoff, Wasserstoff und Schwefel enthalten sind.

Die mit Aceton extrahierten Krystalle sind in Alkohol und Aether sehr wenig, in Aceton, Chloroform und Essigester etwas leichter löslich. In Wasser, Methylalkohol, Benzol, Ligroin und Toluol sind sie unlöslich. Sie enthalten weder Stickstoff noch Halogen. Wenn sie mit metallischem Natrium geglüht und der Rückstand in Wasser gelöst wird, so gibt die Lösung mit Nitroprussidnatrium schöne rote Färbung oder mit Bleiacetat und Essigsäure eine schwarze Fällung. Damit ist die Anwesenheit des Schwefels nachgewiesen. Im Kapillarrohr erhitzt, wird die Substanz gegen 150° allmählich braun und zersetzt sich bei 172° (uncorr.) Nach Literaturangabe ist der Schmelzpunkt von $(CH_3S)_2Hg$ 175° und von $(C_2H_5S)_2Hg$ 86°.

Wird diese Substanz mit konzentrierter Salzsäure erwärmt und das dadurch freiwerdende Gas in Alkohol eingeleitet, so wird es vollständig vom Alkohol aufgenommen. Diese Lösung riecht stark nach Mercaptan und gibt folgende Reaktionen:

1.) Mit Nitroprussidnatrium und Alkali; Rotfärbung, die durch Zusatz von Säuren verschwindet und durch Alkali wieder zum Vorschein kommt.
2.) Mit Isatin und Schwefelsäure; rote Färbung.
3.) Mit Ammoniak und verdünntem Eisenchlorid; rotbraune Färbung.
4.) Mit wässerige Quecksilbercyanidlösung; weisse Fällung.
5.) Mit Alkali und Bleiacetat; gelber Niederschlag.
6.) Mit Quecksilberchlorid; weisse Fällung.

Alle diese Reaktionen sind charakteristisch für Mercaptan. Zur Analyse wurde die Substanz in Vacuo bei 80° getrocknet.

Analyse dieser Substanz (mit Bleichromat verbrannt.)

Substanz(g.)	CO_2(g)	H_2O(g.)	C(%)	H(%)
0.2476	0.0781	0.0480	8.60	2.17

Quecksilberbestimmung.

Substanz(g.)	HgS(g.)	Hg(%)
0.1358	0.1105	70.16

Zur Quecksilberbestimmung wurde die Substanz mit konz. Salzsäure zerlegt und mit Schwefelwasserstoff behandelt und das Quecksilber als HgS gewogen. (Ad. Schenck u. A. Michaelis. Ber. 21 1501 1888).

Vergleich obiger Analysen zahlen
mit dem Procentgehalt einiger Quecksilbermercaptane.

	C(%)	H(%)	Hg(%)
Gefunden	8.60	2.17	70.16

Berechnet f.	$(CH_3S)_2Hg$	8.15	2.05	68.05
	$(C_2H_5S)_2Hg$	14.88	3.12	62.14
	$(C_3H_7S)_2Hg$	20.53	4.02	57.17

Also stimmt die Analysenzahl mit der des Quecksilbermethylmercaptans überein. Das Vorkommen von Methylmercaptan in frischer Raphanuswurzel ist hiermit sicher festgestellt. Ob ein Teil desselben während der Destillation aus labiler Muttersubstanz gebildet wird, bleibt noch zu entscheiden. Es ist aber kaum zu bezweifeln, dass ein grosser Teil desselben in freiem zustande vorkommt. Aus 40 Kilo frischer Wurzeln wurden 3g. Rohquecksilberverbindungen und hieraus 1g. reines Quecksilbermethylmercaptan isoliert. Auf freies Mercaptan berechnet, entspräche es 0.32g.

On the Buck Wheat Protein (Fagopyrum esculentum Moench.) and its Nutritive Value.

Minoru HARA.

(In the Labolatory of the Nutrition Institute, Tokyo, Japan.)

(Received May 10th, 1925)

The three different samples of carefully prepared Japanese buck wheat flour were analyzed into their general constituents as follows:

Sample no.	I	II	III
H_2O	11.965%	12.432%	12.645%
Drymatter	88.035	87.568	87.355
Drymatter%			
Cr. protein (N×6.25)	13 606	13.337	14.250
Protein (〃)	10.250	10.019	9.381
Cr. fat	2.964	2.889	2.986
Cr. ash	2.276	2.278	2.623
N free extract	80.161	80.515	78.934
Cr. fibre	1.003	0.981	1.207
Total N	2.177	2.134	2.280
Alb. N	1.640	1.603	1.549
Non alb. N	0.537	0.531	0.731

In showing the characters of the buck wheat nitrogenous substances on the basis of solubility under different conditions, there are two processes; one is a fractional extraction method of protein and the another an individual extraction method.

The procedure of the former is as follows :– 20g. of the flour is extracted in 500c.c. aqueous solution under agitation for 6 hours at room temperature and after being filtered, 300c.c. of water is added again to the residual, repeating agitation and filtration, then all filtrates are made up to 1 L and estimated nitrogen content by Kjeldahl's method; 10% saline solution is secondarily added to the previons residue and treated in the same manner as stated above, at last time 0.2% NaOH solution is pursued in the same way, nitrogens estimated in each portion are as follows:

	Sample no. I	III
Water soluble N	30.75%	40.63%
10% NaCl soluble N	5.35	4.61
0.2% NaOH soluble N	45.40	40.27
N remained in residue	18.50	14.48
Total N	100.00	100.00

The procedure of the latter consists in extracting samples with aqueous solution, 10% saline solution, 0.2% alkaline solution and 70% alcohol solution separately. Especially in the case of alcohol extraction, temperature is constantly kept at 60–70°C, filtration and estimation is carried out principally following the former process. The results of nitrogen determination is given as follows:

	Sample no. I	II	III
Water soluble N	28.72%	21.27%	39.63%
10% NaCl soluble N	48.07	38.99	43.39
0.2% NaOH soluble N	81.89	78.81	61.53
70% alcohol soluble N	5.01	2.52	2.72
Total N	100.00	100.00	100.00

Above data show that alkaline solution could extract a main bulk of nitrogen from the flour but alcohol solution only a small quantity.

Properties of the extractions:

Solutions extracted by those solvents except alcohol give all protein colour reactions and have more or less viscous character, particularly being conspicuous in the case of alkaline solution which could not be easily filtered out in the clear state.

From the water extraction the coagurable protein is separated by heating at 60–70°C and adding dilute acetic acid and the protein thus obtained yielded as follows:

	Sample no. I
Airdrymatter obtained from original flour 100g.	3.35g.
N yielded for total N of flour	18.50% as drymatter
N content in this protein	13.95% 〃 〃

From the extraction of saline solution, globuline is separated by ordinary method of saturating with ammonium sulphate and is shown as follows:

	Sample no. I
Airdrymatter obtained from flour 100g.	1.85g.
N yielded for total N of flour	13.50% (drymatter)
N content in globuline	15.50% (〃)

The aqueous alkali soluble protein is treated out of extraction obtaided by afore mentioned procedure as follows:- from extracted solution, protein substance is precipitated by adding acetic acid drop by drop as far as solution becomes faintly acid. This precipitate is filtered and redissolved in alkaline solution, and after repeating precipitation and filtration the precipitate is washed by water, alcohol and ether, and dried. This crude protein is a dark brown substance and may be considered to contain glutelin chiefly and is to be albumin and globulin partly; but due to the difficulties of purification of individual simple protein, it is not easy to decide how much the latter two would or would not be actually involved in this substance.

Cotents of water, ash and nitrogen and yield of this substance are as follows:

Sample no.	I	II	III
H_2O	10.72%	4.98%	11.50%
N (drymatter)	14.874	15.820	13.481
Ash (〃)	0.74	0.84	1.08
P_2O_5 (〃)	0.36	—	—
Tryptophane (separated)	0.4	—	—
Airdrymatter obtained from flour 100g.	8.57g.	9.79g.	8.19g.
N yielded for total N of flour	60%	70%	49%(drymatter)

Nitrogen distribution of several groups of aminoacids is determined from this substance by Van Slyke's method as follows:

Sample no.	I		II	
	drymatter(%)	total N(%)	drymatter(%)	total N(%)
Total N	14.892	100.00	15.820	100.00
20% HCl sol. N	14.352	96.37	14.958	94.55
〃 〃 insol. N	0.526	3.53	0.843	5.33
Fumin N	0.151	1.01	0.187	1.18
Amide N	1.679	11.28	1.167	7.38
Phosphotungstates N	4.586	30.80	4.654	29.42
Arginine N	2.247	15.09	2.228	14.08
Lysine N	0.732	4.92	0.830	5.26
Histidine N	1.416	9.51	1.371	8.67

Cystine N	0.191	1.12	0.225	1.42
Amino N	1.957	13.14	2.070	13.09
Nonamino N	2.629	17.66	2.584	16.33
Monoamino N	7.936	53.29	8.950	56.58

Investigation of nutritive value of protein.

For experiments aqueous alkali soluble protein is served as protein source, on account of its comparatively large yield, and casesn is used as contrast.

Animals——Albino rat. 4 (♂) and 4 (♀).

Rations——Ratio of food mixtures as follows:

Ration no.	1	2	3	4
Protein	20%	15%	10%	(no. 3) + (buck wheat flour)
Butter	14	14	14	
Starch	58	65	70	
Nelson's saltmixture.	6	4	4	
Agar	2	2	2	

N. B. Vitamin A, B and C are supplied from butter, oryzanin and cabbage with this ration.

Periods——From March 20 to Dec. 14.1924 (239 days totally).

Summary :–

1. Buck wheat flour contains a large portion of aqueous alkali soluble protein and small amount of coagurable protein and globulin, but none of alcohol soluble protein. All the extractive solution except that of alcohol show viscous character.

2. Aqueous alkali soluble protein contains a large quantity of arginine and histidine.

3. Inspite of the denatured protein the aqueous alkali soluble protein can be regarded, to be a fairly preferable nutritive material better than casein as vegetable protein.

(Dec. 15, 1924.)

On Stereoisomerism of α-Aminocamphor.

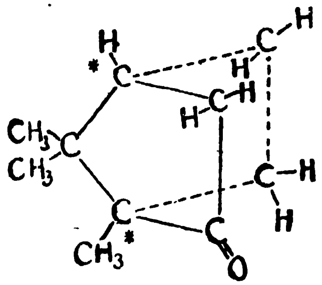
By Yoshikazu SAHASHI.

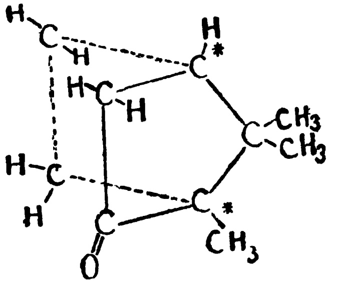
(Received May 15th, 1925)

This paper deals with the results of the work that the author has made under the supervision of Dr. I. Odaira at the Mitsuwa Chemical Intitute, Tokyo.

In 1905, J. Bredt gave the following stereoconfigurations for Japan camphor, and also explained many kinds of the derivatives by means of the formulae.

d-camphor





l-camphor

According to his formulae, both d- and l-camphors will give two kinds (endo and exo) of α-amino derivatives, respectively; but it seems that no stereochemical investigation on primary α-aminocamphors has been attempted. A few years ago, Dr. Odaira proved that Dr. Duden's α- and β-aminoborneols were trans- and cisisomers, respectively, for the benzoyl radical of N-benzoyl β-aminoborneol was able to be transformed to the hydroxyl group in acid solution. However, he did not decide the absolute position of both amino- and hydroxyl-groups in the camphor formula. Consequently, the author thought that the stereochemical investigations of α-aminocamphors would be very desirable for the purpose of the explaination of this problem.

In 1893, L. Claisen synthesized an α-aminocamphor from isonitrosocamphor; and in 1880, Robert Schiff prepared an α-camphor from α′-nitrocamphor; but the further investigations on the stereochemical purification and identification had remained untouched until the author has investigated them by preparing these substances by Claisen's and Schiff's methods and showed that the two compounds were the same one by the melting point and specific rotation of N-benzoylaminocamphor, but even could not succeed in the isolation of the two stereochemical isomers of this substance in pure state. Moreover, the author prepared the hydrochloride, sulphate, picrate and also N-benzoylamino derivatives of α-aminocamphor from isonitrosocamphor or α–brom α'–nitrocamphor by means of different reducing agents such as acetic acid and zink dust, alkali and zink dust, sodium amalgam &c. according to different conditions; and thus the pure substances obtained gave the evidence of constant melting point and specific rotation.

Though the author could not succeed in the separation of the two stereoisomers, he has concluded that the relation between α– and α'– (endo– and exo–) aminocamphors will probably due to the analogous properties exhibited between α– and α'–bromocamphors, between α– and α'–chlorocamphors, or between α– and β–glucoses.

JOURNAL OF THE AGRICULTURAL CHEMICAL SOCIETY OF JAPAN.

Vol. 1, No. 13. October, 1925. No. 13.

ABSTRACTS FROM THE ORIGINAL PAPERS.

Studies on Proteins. (*III*).

Alkali Caseinates

By. Kinsuke KONDO.

(Contribution No. 3. from the Laboratory of Nutritional Chemistry, Department of Agriculture, Kyoto Imp. University) (Received sept. 13th 1925)

A. In this section the author made a short discussion of the works on alkali caseinates which already done by Laquer and Sackur, Robertson and Pauli. Examining these results, we can find that the alkali caseinate solutions employed by them are not able to hold the constant physico-chemical nature even during the experiment. It is therefore, not to be wondered at that the conclusions hitherto done on this problem present a motley picture. In the author's opinion, such simple views of the constitution of protein solutions as those based on the Ostwald law of dilution, for instance, can hardly survive the test of experiment; we may indeed, assert that measurements of conductivity, in the present state of of our knowledge as to theory of solution, are the most difficult of all physico-chemical measurements to deal with, with the sole exception of viscosity measurements.

Nevertheless, it is possible that a few measurements I have made, partly of viscosity, and partly of conductivity in casein solution, may be of some interest.

B. The method used for the preparation of alkali caseinate solution was described. By this method we can prepare the casein solution, which is not only constant and reproducible, but also does not alter their condition in course of time, as long as the quantity of base added is small. This is confirmed by determination of viscosity and conductivity in the solutions. Moreover, the determination of PaH-value and viscosity in alkali caseinate solutions of different concentration of base suggests that the casein is decomposed by the only excess of base, which is confirmed by determinations of formol-titrable nitrogen. These are contrasted with those of Pauli.

C. Under presupposition that we may perhaps follow Pauli in comparing the alkali caseinate solution with a solution of a typical neutral salt, we calculate the quantity $a=\frac{M}{M_\infty}$, and the mobility of casein ions which results 50.45 in the units of equivalent conductivity. The high value a distinctly suggests that the alkali caseinate is a completely dissociated electrolyte. This confirms the opinions of J. Loeb, W. Pauli, S. P. L. Sörensen.

D. (a) Determination of the base-binding power of casein on addition of NaCl and NaOH in varying quantities, revealed a rise in the activity of hydrogen ions with the concentration of sodium chloride with constant quantity of base. This is analogous for instance, to the behavior of acetic acid under similar conditions, and can at any rate in part, be explaind by the activity theory.

(b) Measurements of osmotic pressure and membrane potential in sodium caseinate solutions reveal an extremely high degree of influences exerted by sodium chloride on both osmotic pressure and membrane potential. A brief reproduction is given of a theory for the relation between concentration of sodium chloride, osmotic pressure and membrane potential, a theory closely allied to that of Donnan, and with a very restricted sphere of validity, but nevertheless seeming to show that the molecular weight of casein is very high.

(July, 2nd 1925.)

The Behaviour of Proteases on Amylases, in special Reference to the Chemical Nature of Amylases.

By Shokichi KATO.

(Received, Sept. 15th, 1925)

It is very difficult to know to which class of chemical bodies any enzyme belongs, by direct chemical analysis even of the purest preparations known as yet. The action of one enzyme to another might, however, give us some clew in deciding this question.

Puplications dealing with the behaviour of proteases on amylases, thitherto appeared, have agreed in that the latter is easily decomposed by pepsin, though the action of trypsin is very doubtful.

The author has entered on this study to decide the questions, (1) what is the real cause of the inactivation of amylases by the action of pepsin, (2) how act koji-protease and Bac. subtilis-protease on amylases, (3) whether different amylases are similarly acted by any protease, and (4) whether any evidence can be obtained in concerning with the chemical nature of amylases.

The summary of the experimental results may be given as follows.

1) Salivary amylase is stable at nearly neutral reaction, but easily inactivated irreversirely at the acid side of p_H about 4.2, irrespective of the presence of pepsin (or any protease).

2) In an insufficiently buffered acidic medium containing digestible protein, the acidity increares, as the peptic digestion proceeds. The inactivation of salivary amylase, in such a case, is caused or accelerated by the high concentration of hydrogen-ion, apparently by the direct action of pepsin.

3) But, when the medium is buffered heavily, pepsin shows no effect upon the inactivation of salivary amylase.

4) Salivary amylase is inactivated by none of trypsin, koji-protease and Bac. subtilis-protease even at the resctions nearly optimal for respective protease.

5) The above statements may be given similary on koji-amylase, so far as the experiments are concerned.

6) In short, amylases are not destroyed by proteases. It was perhaps natural that the workers, who did not take into account the above mentioned influence of acidity upon amylases mere led to an errorous conduction.

7) The fact that amylases are indifferent to proteolytic action may be regarded as one of the evidences on the non-protein nature of amylases, though it may not be conclusive.

On Vitamin-A in Green Tea.

by Raizo YAMAMOTO.

(Received, 9th Nov. 1925)

The author observed that green tea contains a fairly large amount of vitamin–A, while black tea almost lacks this complex.

For the isolation of vitamin–A, 1 kg. of finely powdered sample was mixed with 2500 c.c. of a 10% alcoholic potash solution and let it stand for 4–5 days at ordinary room temperature, being stirred from time to time. It was then filtered, and the filtrate was mixed with 2500 c.c. of a 15% alcoholic $CaCl_2$ solution, the precipitate thus formed was filtered off, and carbonic acid gas was passed into the filtrate to remove the excess of calcium hydroxide as calcium carbonate, which carried down colouring matters and other impurities with it. A brown coloured solution obtained by filtering off the carbonate was evaporated under diminished pressure. The residue therefrom was extracted with benzene (B. P. 40–50°); the benzene solution was washed with dilute hydrochloric acid, caustic potash, and water, successively; and dehydrated with dry sodium sulphate. On evaporating off the solvent, a brown coloured syrup was obtained, which possessed a high potency of vitamin–A. A daily dosis of 0,0125–0.0065 gram of this substance was sufficient to induce the normal growth of albino rats. For the curative purpose, twice as much was required. The yield of the raw syrup was 0.1–0.2% of the original sample. It contained still some carotin and other impurities.

The raw syrup gave almost the same colour reaction as "Biosterin," i. e. it gave the characteristic blue colouration with conc. H_2SO_4, Fuller's earth, or with Jendrassik's reagent etc. It acted upon the photographic dry plate in perfect darkness, which property was also shown by original green tea, though in far weeker degree.

The author observed also that the green tea, when caffeine and tannine were removed, was still effective in promoting the growth of albino rats.

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Vol. 1, No. 14. November, 1925. No. 14.

ABSTRACTS FROM THE ORIGINAL PAPERS.

On the Enzymes contained in the Sap of Ficus retusa.

By Syôsuke TANAKA.

(Received, Oct 21, 1925.)

As a result of my experiments the sap of Ficus retusa contains two kinds of enzymes :– the oxidising and the proteolytic enzymes. Among the oxidising enzymes there have been qualified ; oxidase, peroxidase and katalase ; and the proteolytic ones contained, besides the coagulating enzyme, an enzyme like trypsin which acts upon proteins and decomposes it into peptone or albumose and finally converts them to amino compounds, in acid as well as in alkaline solutions.

Vitamin-B Requirement of Different Kinds of Yeast.

By Raizo YAMAMOTO.

(Received, Oct 15, 1925.)

I. The author carried out a series of experiments to see whether there are some chemically defined substances besides vitamin–B or Bios, which accelerate the growth of yeasts. The substances tested were as follows :–

chlorophyll–a, chlorophyll–b, haemoglobin, haemin, quercetin, myricitrin, flavon of green tea, colouriug matter of the cocoon of anthreae yamamai, tannic acid, gallic acid, pyrogallic acid, phloroglucin, vanillin, pyrrol, pyridin, quinolin, furfurol, camphor, camphoroxime, cinnamic alcohol, cinnamic aldehyde, anisic aldehyde, benzaldehyde, nitrobenzene, o-nitro-naphthalene, picric acid unknown picrate from oryzanin, adenin and its picrate, nicotinic acid, β-acid, ethyl-and methyl-ester of β-acid, sodium salt of β-acid, adenyl-thiomethyl pentose, sulphuramino acid ($C_5H_{11}SNO_3$), cysitin, cysteine, cystin plus glutamin, tryptophane, uric acid, caffein semicarbazid, resin, resin (decomposed product of oryzanin), persimmon juice.

The yeasts used for the experiments were :-

Beer yeast Kirin, Wine yeast Nagasawa, Sake yeast (4-5 varieties), and distillery yeast. These were previously cultured on koji-agar.

As the liquid culture media, the Hayduch's and Nägeli's solution were used. The materials used for the preparation of these media were purified with atmost care, especially the cane sugar was repeatedly crystallized until it gave no blue colouration with ferri-ferri cyanid reagent.

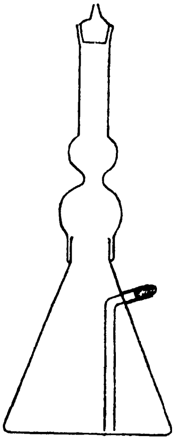
The samples to be tested were added to the culture medium in the extent of 0.01-0.02%. One small loop of the yeast was distributed in 10c.c. of sterilized water, from which 2-3 loops were taken and introduced in each 10c.c. of the above named culture solution. For the control, koji- and malt-juice as well as the medium containing 0.01% of oryzanin were treated exactly in the same way, as above mentioned.

The inoculated solutions were kept at 28°C and the multiplication of the cells and the state of fermentation were carefully observed. When the fermentation was finished, the volume of the yeast, settled at the bottom, was compared with the control one.

The results of the experiments were almost negative. Only β-acid (dioxy-chinolin-carboxylic.acid, obtained by the hydrolysis of Suzuki's crude oryzanin) its salts and esters exhibited a slight stimulating action upon beer and wine yeasts, though its action was far weaker than oryzanin itself. Picric acid was found to be slightly efficacious for the sake yeast.

II. It is well known that vitamin-B accelerates the growth and fermentative power of yeasts, though different kinds of yeasts behave quite differently towards it. Thus certain yeast growing at the bottom of the culture solution require much vitamin-B, while others growing on the surface of the same medium are found to be less influenced by it. The author made careful studies on this subject with the following 32 kinds (species or varieties) of yeasts, i. e.

- Saccharomyces Sake ; 7 varieties
- Saccharomyces Cerevisiae ; 5 〃
  - Beer yeast Kirin
  - 〃 〃 Yebisu
  - 〃 〃 Frohberg
  - 〃 〃 Saaz (Unter-gärige)
- Saccharomyces Ellipseudius 6 varieties
  - Wine yeast Nagasawa
  - 〃 〃 Yamanashi
  - 〃 〃 Johanisberg
  - 〃 〃 Oppenheimer
  - 〃 〃 Charente Champagne
  - 〃 〃 Albo
- Saccharomyces Shausing 5 varieties
  - No. I
  - 〃 II
  - 〃 II (yellow)
  - 〃 III
  - 〃 IV
- Distillery yeast
- Saccharomyces Exigus
- 〃 Pasteurianus
- 〃 Ellipseudius
- Zygosaccharomyces Major
- 〃 Soja
- Willia Anomala
- Pichia Farinosa
- 〃 Rosa



As the source of vitamin-B, commercial "oryzanin" liquid (20 % alcoholic solution) was used, of which 0.005, 0.01 and 0.02mg. were added per c.c. of the culture medium.

For the determination of the fermentative activity, a special fermentation flask, constructed by Dr. U. Suzuki was used (J. Chem. Soc. Japan Vol. XLV, Nos. 5 & 6. June, 1925) It is a narrow necked flask of 100c.c. capacity, connected with a vertical $CaCl_2$ -tube the CO_2 evolved escapes through the tube, while the moisture being retained in it. On the upper side of the flask, there is a narrow bent tube, one end of which reaches nearly to the bottom of the flask, while the other end opens outside of the flask and is stoppered with rubber. This tube serves for the inoculation of yeast. (See Fig. I.) Each 50c.c. of the culture solution were filled in the flask, and 1c.c. of a dilute yeast suspension to be tested was inoculated to it. The flask was now kept in a thermostatt at 28°. During the experiment the whole apparatus being weighed at definite intervals of time. The decrease in weight corresponds to the quantity of CO_2 evolved. Thus the percentage of sugar consumed by fermentation may be calculated and plotted in a curve.

Comparing these curves, the author observed the following facts:

1. Different varieties of sake yeast grew very rapidly in the medium containing 0.01 % of oryzanin, and decomposed the sugar almost completely in 7 days, while in the control medium containing no oryzanin, the growth was hardly visible until 5–6 days, but after that time they grew at the same rate with the former one. Sake yeast seems therefore to require only very little vitamin–B (if any) for their growth.

2. Beer yeasts grew generally very slowly in the vitamin free medium, only 1–5 % of the total sugar being decomposed in 2–3 weeks. By the addition of oryzanin, the growth was accelerated nearly in proportion to the amount of it added. They belong therefore to the class, which requires much vitamin–B.

3. Wine yeasts stand in the middle of sake and beer yeasts in this respect.

4. Distillery yeasts also required vitamin–B in fairly large amount.

5. Saccharomyces Shausing, which grows on the surface of the calture medium and forms scum on it, was found to be less influenced by vitamin–B.

6. Willia anomala grew well in the vitamin free solution, but the growth was somewhat better in presence of vitamin.

7. Zygosaccharomyces major and soja hardly grew even in the medium containing 0.02 % of oryzanin, but grew in koji or malt juice and strongly fermented, this variety therefore seems to require much vitamin.

8. Pichia rosa and farinosa did not require much vitamin.

From these results it may be concluded that the cultivated bottom yeasts generally require more vitamin for growth than the wild, top growing kinds do.

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